

ATMOSPHERE STABILIZATION AND ELEMENT RECYCLE
IN AN EXPERIMENTAL MOUSE-ALGAL SYSTEM

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IN AN EXPERIMENTAL MOUSE-ALGAL SYSTEM

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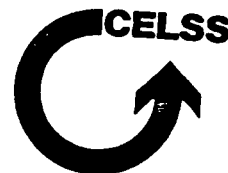


TABLE OF CONTENTS

List of Figures	ii
List of Tables	iv
Chapter I INTRODUCTION	
Background	1
Importance and Originality	6
Statement of the Problem	7
Issues to be addressed	9
Limitations and Assumptions	11
Organization and Methodology	12
Chapter II LITERATURE REVIEW	
Gas Exchange	13
Waste Processing	21
BLS Concepts	
General Issues	24
Food Production	26
Control Issues	29
Chapter III MATERIALS AND METHODS	
Mouse Reactor	32
Algal Reactor	34
Control System	36
Wet-Air Oxidation	37
Experimental Methods	38
Chapter IV DATA ANALYSIS AND DISCUSSION	
Atmosphere Stabilization	
Respiratory Quotients	42
Assimilatory Quotients	42
Combined Algal-Mouse Experiments	48
Mass Balance	61
Growth Experiments	76
Chapter V CONCLUSIONS	82
REFERENCES	88
APPENDIX A	92
APPENDIX B	94

List of Figures

<u>Figure</u>	<u>Title</u>	<u>Page</u>
1	Comparison of Crew Life Support Options	2
2	Cumulative Mass Launched vs. Mission Time for Life Support Options	2
3	Material Cycling Within a CELSS (simple)	4
4	Material Cycling Within a CELSS (complex)	4
5	Experimental Gas-Closed Mouse-Algal System	8
6	Experimental Gas-Closed Mouse-Algal-Wet Oxidizer System	8
7	Stoichiometry of Gas Exchange	15
8	Experimental Mouse-Algal System	33
9	CO ₂ -O ₂ Relationship in a Gas-Closed Mouse System	43
10	CO ₂ -O ₂ Relationship in a Gas-Closed Algal System	43
11	Variation of Assimilatory Quotient as a Function of Optical Density	45
12	CO ₂ -O ₂ Relationship in a Gas-Closed Mouse-Algal System (nitrate)	50
13	CO ₂ -O ₂ Relationship in a Gas-Closed Mouse-Algal System (urea)	50
14	CO ₂ -O ₂ Relationship in a Gas-Closed Mouse-Algal System (nitrate)	51
15	CO ₂ -O ₂ Relationship in a Gas-Closed Mouse-Algal System (nitrate)	53
16	CO ₂ -O ₂ Relationship in a Gas-Closed Mouse-Algal System (urea)	53
17	CO ₂ -O ₂ Relationship in a Gas-Closed Mouse-Algal System (nitrate and urea)	55

List of Figures (continued)

<u>Figure</u>	<u>Title</u>	<u>Page</u>
18	Crossover Area as a Function of Optical Density and Light Intensity (nitrate)	57
19	Crossover Area as a Function of Optical Density and Light Intensity (urea)	57
20	Simulation Model; Mouse/Algal/WAO System	62
21	Simulation Model; Mouse Compartment	66
22	Simulation Model; Algal Compartment	69
23	Simulation Model; Wet Air Oxidizer Compartment	71
24	Growth of Algae on Wet Oxidized Mouse Feces	77
25	Growth of Algae on Wet Oxidized Chlorella	80

List of Tables

<u>Table</u>	<u>Title</u>	<u>Page</u>
1	Mass Balance of a Dwarf Mouse	67
2	Wet Air Oxidation Mass Balance	72
3	Mass Balance of the Mouse-Algal-WAO System	74
4	Effect of Salt Concentration on Algal Growth	78
5	Mouse Mass Flow Data	92
6	OD and AQ of Nitrate Grown Chlorella	93

I. INTRODUCTION

Background

One objective of the National Aeronautics and Space Administration (NASA) is to develop a permanently manned space station in Earth orbit. Future United States space policy may include Lunar and Martian bases and construction of orbiting habitats. As mission duration and crew size increase regenerative life support technologies will be required. Life support technology used to date relies on the storage of water, oxygen and food and the storage or disposal of carbon dioxide and waste materials (Fig. 1). The Controlled Ecological Life Support System (CELSS) is being developed by the Bioregenerative Life Support (BLS) research program to provide resupply-independent life support technology for use in future U.S. manned space missions.

As the number of humans and their length of stay in space increases the feasibility of bioregenerative life support improves (Mason, 1980; Gustan and Vinopal, 1982). The cost and weight savings of a CELSS are directly related to launch costs of expendables as compared to the equipment and maintenance costs associated with a CELSS. The breakeven point for bioregenerative life support varies with mission scenario and the expendable life support technologies to which it is compared. Figure 2 compares the cumulative mass launched for resupply,

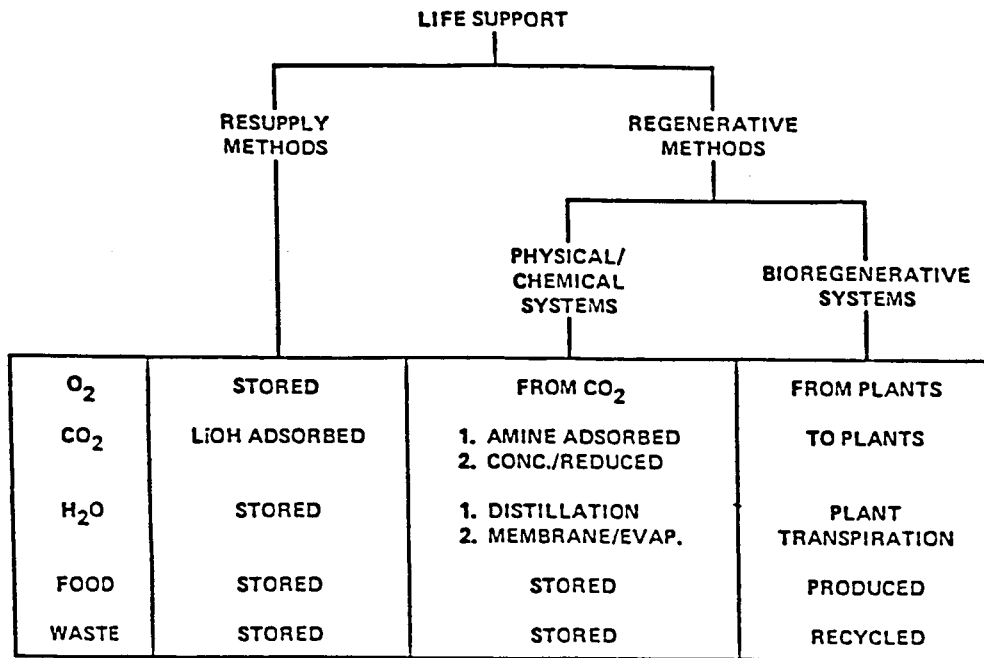
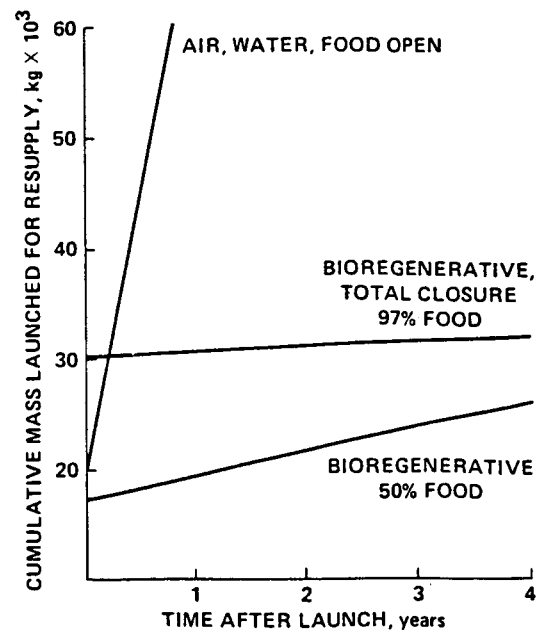


FIGURE 1 Comparison of Crew Life Support Options
(from MacElroy et-al, 1985)

**ESTIMATED BREAKEVEN TIME
MISSION: LEO (4 PERSON)**



**FIGURE 2. Cumulative Mass Launched vs. Mission Time
for Life Support Options**
(from Gustan and Vinopal, 1982)

partial bioregenerative and full bioregenerative life support options. The breakeven time occurs at the intersection of the curves and is the point where the costs of resupply exceed the cost of supplying a bioregenerative system.

The premise underlying a CELSS is to recycle waste materials into supplies required for human survival. A combination of physical-chemical and biological processes will be used to convert CO_2 to O_2 , recycle water and produce food (Fig. 3). Waste materials (feces, urine, wash water, and plant wastes) will be oxidized into biologically useful forms for use in the food production system. Volatile organics and other toxic materials will be removed from the system. Food production will be based on plants and algae which will also absorb CO_2 , produce O_2 and purify water by transpiration. All of these requirements lead to a complex overall system (Fig. 4). A CELSS will thus eliminate or greatly reduce the need for costly resupply of water, oxygen and food and the storage or disposal of waste materials.

In addition to the capacity to recycle materials, a CELSS must demonstrate operational stability. To assure stability and resilience a variety of control mechanisms will be required. Endogenous control mechanisms, i.e. control based on inherent properties of the organisms within the system, are useful because once established they require relatively less input of mass, energy and

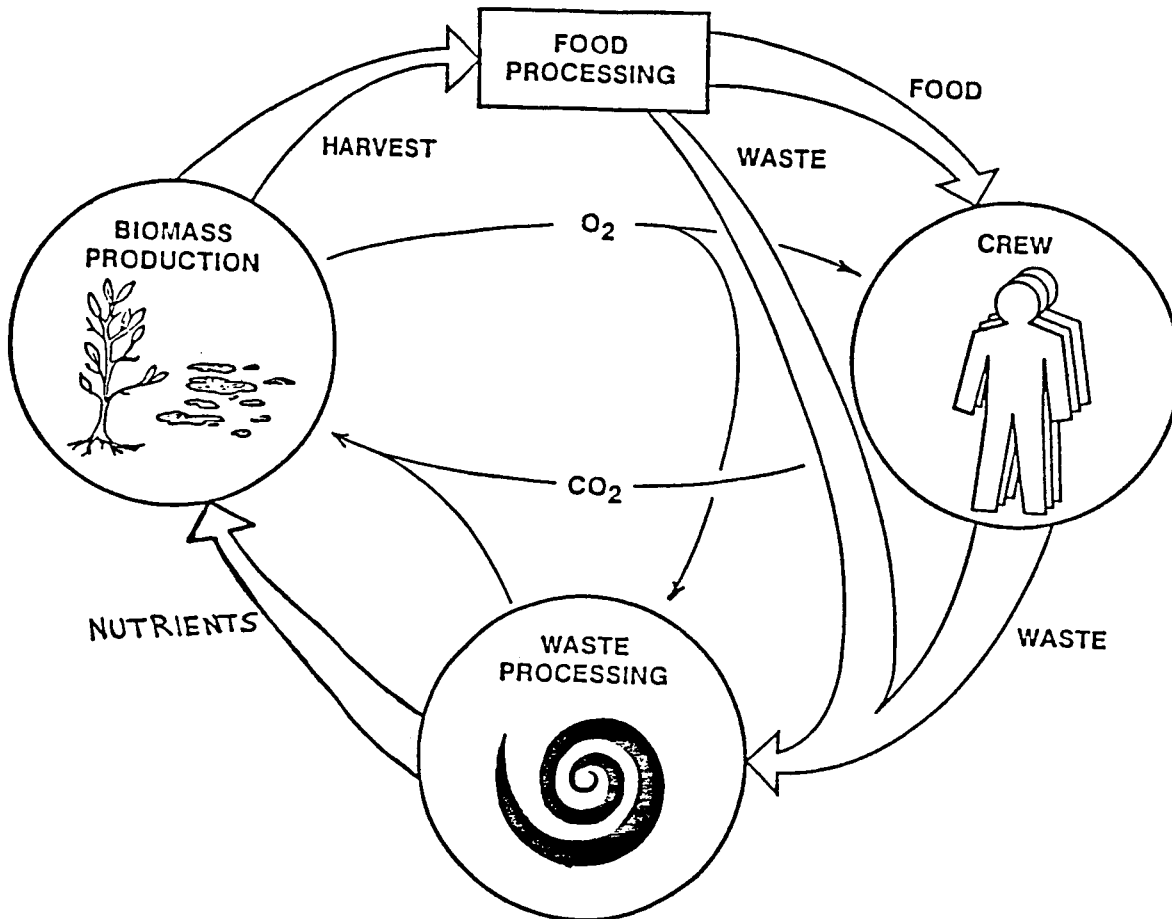


FIGURE 3. Material Cycling within a CELSS (simple)

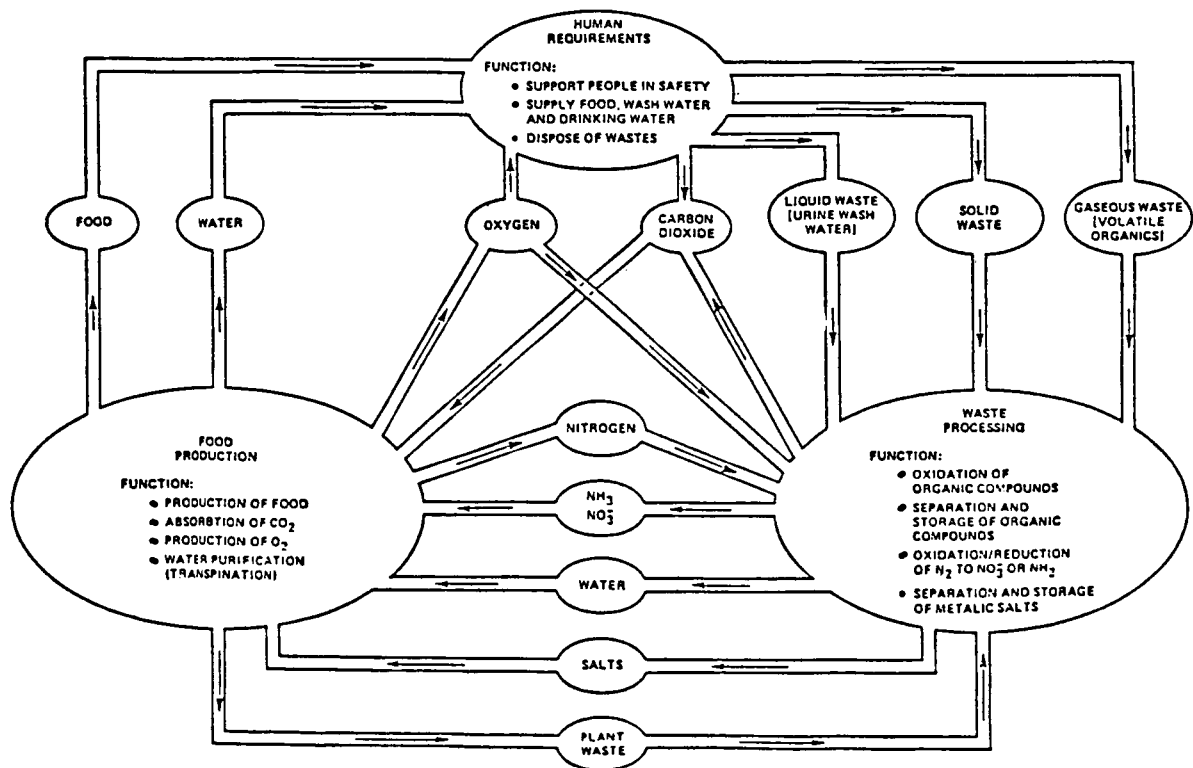


FIGURE 4. Material Cycling within a CELSS (complex)

maintenance. Exogenous control relies upon engineered devices which are more completely understood and allow control to be exerted more easily but at the cost of increased mass, energy and maintenance. Approaches for improvement of endogenous control strategies are addressed by research described in this thesis.

Beyond feasibility and stability a CELSS must demonstrate longevity. There are a host of issues related to the long-term health, and hence survival, of the crew. Detection and removal of trace contaminants in air, water and food; control of indoor radiation levels; presence of appropriate sensory stimulus; and maintenance of a pathogen-free system are issues related to the longevity of a CELSS in space environments. These issues are beyond the scope of this thesis but must be recognized as important to the overall BLS concept.

One goal of the BLS program is to minimize the mass, power and volume required to perform life support tasks. Another is to construct a CELSS that can recycle most or all essential materials in sufficient quantity and at the proper rates to support crew requirements. Theoretical data indicates that such systems can be operated, experimental verification is now required to support theory and develop required technologies (Moore, Wharton and MacElroy, 1982).

Importance and Originality

The need for bioregenerative life support technology rests primarily on the economic advantage which a CELSS could provide to manned space missions. This thesis explores certain aspects of BLS concepts which have not yet been studied experimentally. The specific areas to be studied fall into two categories. Primary is the ability to create a stable, photosynthetic gas exchanger amenable to control as required by the crew simulator (mouse). The other is to quantify bioelement (carbon, oxygen, hydrogen and nitrogen) flows through the experimental systems compartments (mouse, alga and waste processor).

Experimental systems are useful for simplifying CELSS functions. They allow quantification of material and energy flows and for testing the accuracy of computer models. The experimental system described in this thesis is based on mice (dwarf strain DW/J) and algae (Chlorella pyrenoidosa) and was built at NASA-Ames Research Center to study principles of photosynthetic atmosphere regeneration. Figure 5 shows the components of the atmospheric portion experimental system. In addition to gas exchange experiments the system provides insight into the use of inherent properties of organisms as system control points.

Experimental data has been gathered for the flow of materials (food, water, feces and urine) through the mouse compartment. Waste products from both algal and mouse compartments have been oxidized in an abiotic waste processor. At this time the mouse-algal part of the system is not physically coupled to the waste processor although flows between the compartments are being investigated. Figure 6 shows the components of the overall experimental system and indicates routes of mass transfer that are not physically linked. Further fundamental research is required to achieve elemental balance of bioelements through the system. The importance of work described in this thesis lies in establishing principles and control strategies for photosynthetic atmosphere revitalization and for studying mass closure within simple closed systems.

Statement of the Problem

How might stable CO₂ and O₂ concentrations be maintained within an experimental gas-closed mouse-algal system? What results of element recycle experiments are useful for determining mass closure within the experimental system?

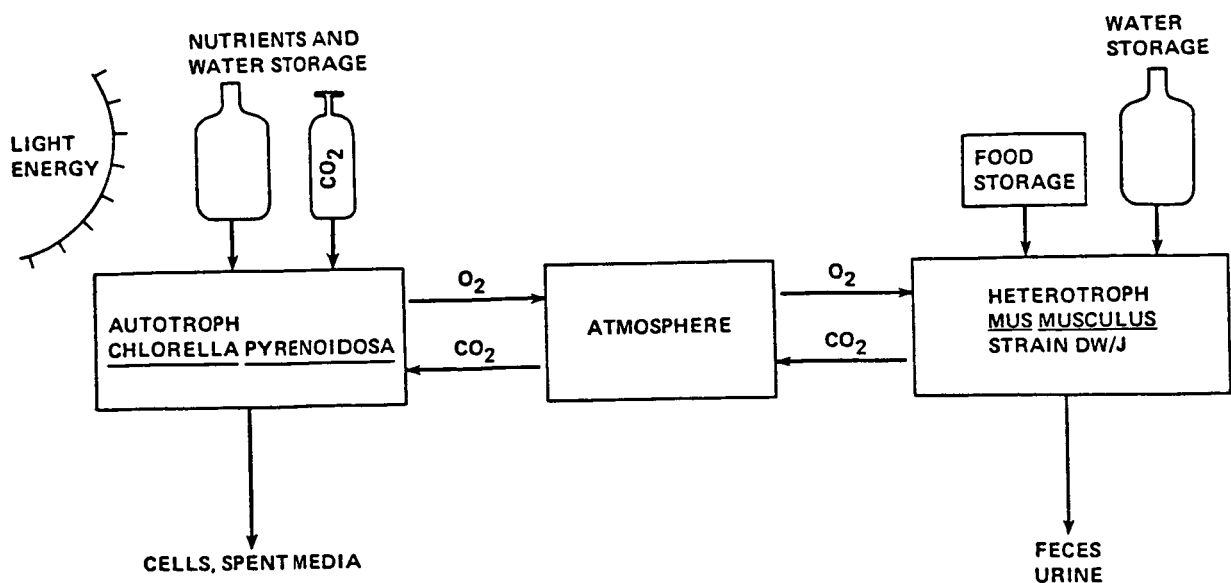


FIGURE 5. EXPERIMENTAL GAS-CLOSED MOUSE-ALGAL SYSTEM

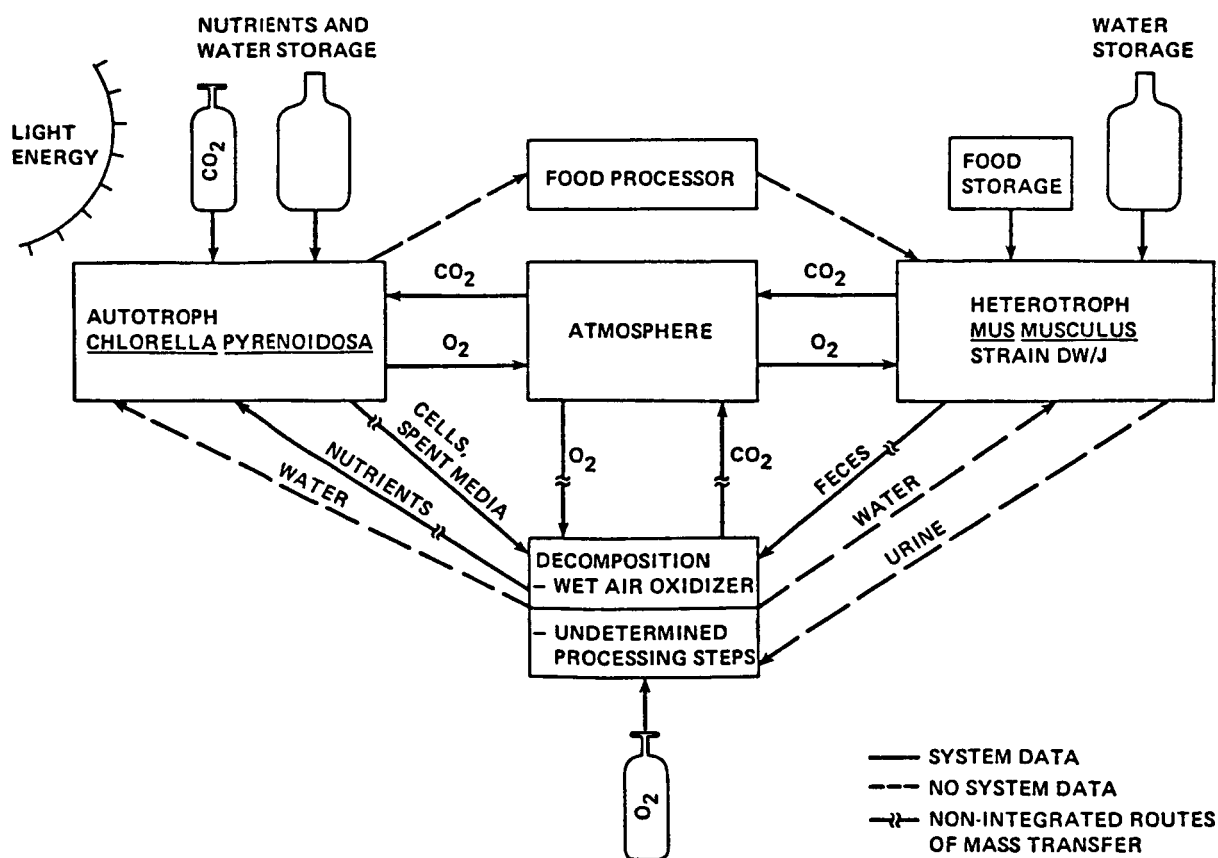


FIGURE 6. EXPERIMENTAL GAS-CLOSED MOUSE-ALGAL-WET OXIDIZER SYSTEM

Issues to be addressed

- I. How can the dynamics of CO₂ and O₂ exchange within the experimental CELSS gas-closed mouse-algal system be manipulated to achieve stability?
 - A. What dynamics of CO₂ and O₂ exchange are observed as the system is operated with the algal and mouse compartments separate?
 - B. What CO₂ and O₂ exchange dynamics are observed when the system is run with the algal and mouse compartments coupled?
 - C. How do algal growth rate manipulations (i.e. variation of light intensity and cell density) affect the CO₂ and O₂ exchange dynamics?
 - D. How do manipulations of the algal AQ ratio (i.e. variation of nitrogen source) affect CO₂ and O₂ exchange dynamics?
 - E. Do combinations of rate and ratio manipulations allow long-term (days) maintenance of stable CO₂ and O₂ concentrations?
- II. How do contaminant microbial populations affect the relative contributions of photosynthesis and respiration into the atmosphere of the system?

- A. How significant is the bacterial contamination of the algal cultures and/or the mouse feces?
- B. What order of magnitude are the rates of respiration of the mouse and of the contaminant bacterial populations within the system?
- C. How can contaminant bacterial respiration be minimized to avoid interference with the atmosphere dynamics of the mouse-algal interaction?

III. What conclusions concerning mass flows within the system can be made from initial element recycling experiments?

- A. What element flows have been quantified through the mouse reactor?
- B. To what extent has element balance been achieved through the abiotic waste oxidizer?
- C. How well has the waste processor oxidate supported algal growth?
- D. Does preliminary work indicate that the algal reactors nutrient requirements can be met solely from processed waste materials?

- E. To what extent has bioelement balance been achieved through the experimental system?

Limitations and Assumptions

The experimental system is operated with several simplifying assumptions about material and gas flows. The dissolved CO₂ and O₂ in the reactors is not measured and is assumed to remain within narrow ranges due to pH stability. The emission of trace volatile gases from either system components or organisms is assumed to have no significant short-term effect on the metabolic functions of either the mouse or algae. In atmosphere recycling experiments it is assumed that the mouse adequately represents human physiologic characteristics. It is also assumed that the culture techniques and algal species selected for an operational CELSS will not significantly alter atmosphere exchange dynamics observed with the experimental system. Problems associated with gas dispersion and radiation effects in micro-gravity (micro-g); algal productivity; light use efficiency and mass, power and volume optimization are not covered by this research.

The experimental system has several limitations relating to its physical configuration. The system is limited to gas exchange between the mouse and algae with no direct flow of nutrient materials between the system

compartments nor are there physical links between the mouse-algal compartments and the waste processor. This limits the ability to determine the degree of recycling within the system. However, conclusions concerning the flows of elements through these non-integrated portions of the system will be made (Fig. 6).

Organization and Methodology

This study is divided into two sections. First the dynamics and control of gas exchange between the mouse and algae are examined. In order to achieve complete balance of CO_2 and O_2 , the food loop must also be closed, therefore the flow of bioelements through the systems compartments are studied. Conclusions regarding the stabilization of atmospheres and the degree of element closure are made. Finally, these conclusions raise important questions concerning the development of stable, mass-closed ecological systems for use as life support systems in space.

II. LITERATURE REVIEW

Gas Exchange

The first experiments with photosynthetic gas exchange may be attributed to Joseph Priestly who in the late 1700's performed a series of classic experiments demonstrating the exchange of substances between a mouse and a plant. Both the plant and the mouse would die if placed in separate gas-closed chambers, yet if they were placed in a chamber together both survived (Yule, 1978). Although Priestly had no notion of the underlying phenomena that he observed, he named the substance oxygen and opened the doors to further discovery.

Some two hundred years later, in 1963, Eley and Myers performed a set of experiments to quantify the observations of Priestly. They constructed an experimental system to accurately measure the exchange of O_2 and CO_2 between photoautotrophic algae and an heterotrophic mouse. They found that bacterial respiration could significantly impact system dynamics and therefore they designed their system to minimize bacterial contamination. They concluded that a complete balance of CO_2 and O_2 is not possible without matching the respiratory ratio of the mouse to the photosynthetic ratio of the algae (Eley and Meyers, 1964).

The reason for the observed imbalance in the photosynthetic gas exchanger is due to both different rates of photosynthesis and respiration and mismatches between the respiratory quotient (RQ) ($RQ = \text{moles } CO_2 \text{ produced} / \text{moles } O_2 \text{ consumed}$) of the heterotroph and the assimilatory quotient (AQ) ($AQ = \text{moles } CO_2 \text{ consumed} / \text{moles } O_2 \text{ produced}$) of the autotroph. The apparent AQ of an algal system will be a combination of photosynthesis and cellular respiration. Figures 7a and 7b numerically demonstrate how gas concentrations would vary in a gas-closed mouse-algal system in which the algal AQ exceeds the mouse RQ.

Figure 7a demonstrates that if the CO_2 concentration is held constant by control of the algal photosynthetic rate there will be a continual loss of O_2 at the indicated AQ and RQ values. Starting with 1.0 moles of O_2 entering the mouse, 0.85 moles of CO_2 will be produced ($0.85 = X/1.0$; $X = 0.85$). The 0.85 moles of CO_2 enter the algal reactor yielding 0.895 moles of O_2 ($0.95 = 0.85/X$; $X = 0.895$). Thus each cycle of the system will result in a loss of 0.105 moles of O_2 . Similarly, figure 7b shows that if O_2 is held constant by manipulation of the algal photosynthetic rate a loss of 0.10 moles of CO_2 will occur through each cycle. Closer matches between AQ and RQ will result in less depletion of either CO_2 or O_2 from the system.

AQ = 0.95
RQ = 0.85

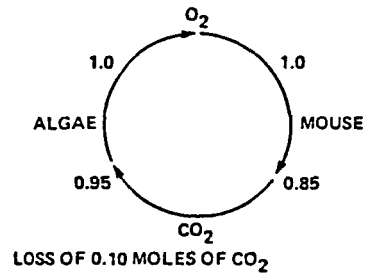
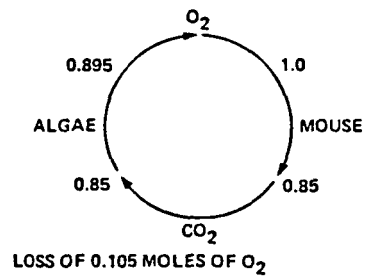


FIGURE 7. Stoichiometry of gas exchange in a gas-closed, mouse-algal system in which either carbon dioxide exchange is balanced (top) or oxygen exchange is balanced (bottom).

Eley and Myers demonstrated that this exchange of gases will have an inherent instability due to mismatches between AQ and RQ of a particular autotroph and heterotroph. Typical algal AQ's range from 0.6 to 0.95 depending on species and nitrogen source. The biochemical reactions which metabolize each nitrogen source vary and require different amounts of CO₂ and energy with concomittant differences in O₂ output. These differences in biosynthetic pathways result in different CO₂ consumption and O₂ production. Typical mouse RQ's range from 0.85 to 0.90 (Eley and Meyers, 1964) although higher values (1.0) have been observed (Averner, 1984a). RQ varies with the nutritional source (carbohydrate, lipid, protein) being metabolized which in turn is affected by the activity level and diet of the mouse.

Extrapolating to a space-based system, to efficiently supply a human crew's oxygen demands and carbon dioxide removal requirements, an algal photosynthetic gas exchanger must match the algal AQ to the crew's varying RQ. It is possible to modify the crews diet or activity levels in order to alter the crew RQ, however, this is not a realistic control option.

Numerous experiments were conducted during the late 1950's and early 1960's concerning gas exchange in gas-closed animal-plant systems. For example, in 1958 Doney and Myers investigated a system with two mice and the alga Chlorella ellipsoidea. The 30-day experiment

showed CO₂ levels vary between 0.5% and 1.0% with O₂ levels rising from 21.0% to 25.0%. They attributed the rise in O₂ to a mismatch between AQ and RQ.

In 1962 London and West attempted a more complex closed ecosystem which included the fungus Liderina pennespora, the alga Chlorella pyrenoidosa, a rat and a sewage reactor. Closure of the system led to rapid changes in CO₂ (0.04% to 12.3%) and O₂ (20.2% to 5.77%) concentrations within 43 hours. Removal of the fungus slowed the rates of change, however no definite conclusions about these experiments can be drawn due to continual manipulation of the system configuration. Nevertheless it is apparent that simple multi-species systems do not establish stable states when operated as free-running systems.

In 1963 Gouleke and Oswald operated a system containing mice, algae and bacteria which demonstrated stability for up to six weeks. A conclusion of their study was that the rate of photosynthesis was a function of the CO₂ output of the mice. Removal of a mouse would lower the CO₂ concentration and effect the algal photosyntheis thereby causing a decline in O₂ production. However, an equilibrium was slowly established in which the O₂ concentration would return to a level close to the original value. This seems to indicate that the system was operating with some endogenous control through the response of photosynthesis

to CO₂ concentration. Eley and Myers (1964) pointed out that the responses seen could have been due to exchange leaks with the outside atmosphere.

One common feature of these experiments and others is the significant fluctuation of the O₂ and CO₂ concentrations within the atmospheres of the systems. Reasons cited for these fluctuations are: differences in rates of photosynthesis and respiration; mismatches between AQ and RQ; bacterial respiration within algal cultures or fecal material; gas leaks between the system and the external atmosphere; and material imbalances within the system (i.e. tie up of oxygen in metabolic water and net flux of carbon and oxygen between inputs (food) and removals (harvested algae)) (Averner, 1982). The bulk of work has been directed at observing fluctuations in gas concentrations within free-running systems. Almost no work has been done on the balancing of AQ and RQ within simple plant-animal systems. The application of endogenous control strategies to regulate such systems is just beginning to be explored by the research described in this thesis.

Experiments conducted in the Soviet Union have outlined strategies for control of gas exchange within closed animal-plant systems (Shabal'nikov, 1975; Nasonov, 1977). Approaches suggested include; use of mixed autotroph populations (e.g. two algal reactors with different AQ's), or to switch between operating regimes

such as light and dark periods. Both Soviet and American research indicates that photosynthetic atmosphere revitalization is quantitatively possible but has not yet been demonstrated experimentally. Systems containing several different autotrophs and heterotrophs may be useful because greater stability might be achieved with multiple AQ's and RQ's. The Soviets have operated a multi-species bioregenerative life support system which includes photosynthetic gas regeneration, however data is not readily available. The apparent success of this system (BIOS) does indicate that multiple species systems, although more complex, may prove to be more stable and amenable to control (Ivanov and Zuberava, 1985).

Gas exchange research ongoing at Ames is focusing on development of strategies for control of algal AQ's. (Averner, 1983; Averner, 1984a). The atmosphere within a closed system in space could be balanced by resupply of gases from storage but the objective of a CELSS is to minimize or preclude the resupply of materials. Extravehicular activities (EVA) and cabin leakage will also effect the atmospheric concentrations of CO₂ and O₂ and will reduce the need for matching AQ to RQ (Humphrey, 1969). However, the ability to control AQ will provide additional stability and reliability to the atmosphere regeneration system. For these reasons it is desirable to develop reliable control mechanisms for exploiting the inherent properties of the photosynthetic

component.

Control strategies under study include variation of the photosynthetic rate by manipulation of environmental parameters such as light intensity, cell density and chamber volume. Such approaches are useful in that they affect rates of CO_2 uptake and O_2 production. Such rate manipulations will be useful in a multi-species system, however they do not alter the AQ and thus will eventually lead to a loss of either CO_2 or O_2 . The partial pressures of CO_2 and O_2 will vary according to both relative rates of photosynthesis and respiration and to the relative values of AQ and RQ. As shown earlier (Fig. 7), if the AQ exceeds the RQ and the CO_2 concentration is held constant by rate manipulation, the O_2 concentration will decrease. Minimization of these losses will reduce the need to stabilize the atmosphere by more active control mechanisms.

Averner (1982) has pointed out that techniques to control the AQ are more difficult than rate control but are more useful because they eliminate ratio instability of mismatched AQ's and RQ's. Use of different nitrogen sources affects algal AQ's. Nitrate (NO_3^-), urea, and to a lesser extent ammonia (NH_4^+), have been shown to exhibit AQ variability. Experiments now in progress use two reactors, each with characteristic AQ's, to achieve greater control of ratio instability. Another technique which may be useful is the use of temperature variation to

effect algal photosynthesis. It is not clear whether this effect is due to AQ ratio changes or to photosynthetic rate changes. Experiments to test this approach have not been done by this research and thus are not included in this thesis.

The Earth is able to maintain short-term steady-state concentrations of CO_2 and O_2 because of the large buffering capacity of the oceans and the atmosphere. Long-term stability is achieved because of the large number of organisms and hence large range of AQ's and RQ's present in the global biosphere. The accumulation of organic matter also plays a role in maintaining steady state concentrations of CO_2 and O_2 . Large buffers, species diversity or accumulation of organic matter will not be possible in a CELSS making a close match of AQ to RQ desirable.

Waste Processing

Conversion of waste materials into useful products is essential to the success of a CELSS (Moore and MacElroy, 1982; Moore, Wharton and MacElroy, 1982). Thus, one goal of the experimental system is to achieve partial elemental balance between the autotroph, heterotroph and waste processor compartments. In order to recycle materials, they must flow from one compartment, through a waste processing subsystem, into another compartment. Figure 6

displays the flows, both integrated and non-integrated, which the materials undergo in this portion of the experimental system.

The experiments described in this thesis rely on the conversion of waste material into an algal nutrient source by a physical-chemical process known as wet-air oxidation (WAO) (Onisko and Wydeven, 1979; Gillen and Olcott, 1974). The primary alternatives considered for waste processing in a CELSS are wet-air oxidation, aerobic biological oxidation and supercritical water oxidation (Shuler, 1979; Shuler, 1981; Ballou, 1982; Modell et al, 1982;) but only WAO has been used in these experiments. The method of choice for oxidizing waste materials in a CELSS will depend on the input stream, the products, the efficiency of the system and the degree of control possible. The determination as to which method or combination of methods will be used in a CELSS is not the objective of this paper. However, the analysis of data generated by; element balance through the WAO reactor and conversion of mouse feces and harvested algal cells to an algal nutrient source is germane. Analysis of data will include; element balance, effectiveness of the WAO process, growth characteristics of algae grown on the products of WAO and inhibitory effects of WAO by-products.

WAO is a well documented, commercial process adapted to accomodate various waste streams (Perkow et al, 1981; Randall, 1981). Work ongoing at Ames Research Center is directed at optimizing the products of WAO using a standardized human waste stream (Wydeven, 1983). The usefulness of any waste processing subsystem in a CELSS will be determined by many factors. Most important among those is the ability of the process to supply a pathogen- and toxin-free nutrient source for use by components of the food production and air revitalization subsystems. Variation of WAO process parameters such as temperature, pressure, residence time and use of catalysts is being studied to tailor the process products to photosynthetic organism growth requirements (Johnson and Wydeven, 1983).

Onisko (1983) demonstrated that the products of the WAO reactor in use at Ames contain elements (boron and silver) toxic to lettuce seedlings. Precautions have been taken to reduce and/or eliminate the sources of that toxicity. Experiments conducted in our laboratory using system waste products (feces and harvested algal cells) and the WAO reactor show the presence of, as yet unidentified, inhibitory compound(s). The data from these experiments will be analyzed to determine if it is feasible to use the systems waste products as the sole source of algal nutrients.

Waste processing technology is also being studied to improve power, mass and volume ratios. For example, nitrogen fixation is highly energy intensive yet can be avoided by using waste processing strategies which do not reduce organic forms of nitrogen to N_2 . The use of nitrogen-fixing species of algae is also being studied (Packer, 1984). Similarly, by minimizing output of toxic materials from waste processing systems additional waste treatment steps can be minimized or avoided. In addition, proper selection of spacecraft materials will reduce the burden on trace contaminant control subsystems. Design of a waste processing subsystem that maximizes output of materials in biologically assimilable forms will reduce the need for secondary systems to convert materials into useful forms.

BLS Concepts

General Issues

The bioregenerative life support program is exploring scientific and technological issues required for development of a functional CELSS. Criteria fall into two categories, those associated with cost, and those related to technical capability. Cost issues are closely tied to the power, mass and volume required to build and maintain a CELSS. Technical issues must address scientific and

engineering questions associated with operation of an artificial ecosystem. Therefore, research efforts are directed not only at demonstrating that water, oxygen and food can be regenerated from carbon dioxide, feces, urine, wash water and plant wastes but that it can be done within a relatively small volume (hence mass) and without extensive power requirements.

Other areas for improving CELSS efficiency involve minimizing buffer and reservoir sizes. The Earth provides enormous buffers (oceans and atmosphere) for storage of materials (gases, water, nutrients) and dilution of toxins. These buffers cannot be duplicated in a CELSS, thus the functions of the buffers must be engineered into the system. Minimizing reservoir sizes and techniques to alter rates of material flow will reduce the cost requirements (i.e. increased volume) of supplying buffer capacity. Additionally, the implementation of robotics will reduce requirements for human intervention into routine tasks such as planting, harvesting and processing of food.

In order to pursue these types of investigations both ground-based and flight experiments will be required (MacElroy, Smernoff and Klein, 1985). Ground-based experiments will focus on recycling issues and problems associated with increasing the size of experimental systems (scale up). Several modules (plant and algal growth, waste processing, crew simulation and control)

will be designed, built and integrated in order to study the behaviour of subsystems and the integrated system. Flight experiments will focus on the growth of plants, algae and bacteria in micro-g. Physiology issues will be addressed relating to micro-g and radiation effects on plants and algae. Engineering issues will deal with separation of liquids and gases in micro-g as well as requirements for operating life support equipment in the space environment.

Food Production

One critical area of CELSS research involves increasing the productivity of selected plant species. The yield of edible biomass per unit area is being increased significantly by the use of controlled environmental conditions (e.g. high CO₂ concentrations, elevated temperatures and specialized nutrient delivery systems). Another related area is concerned with increasing the edible/nonedible biomass ratio. Genetic engineering, improved extraction and processing techniques and synthesis of foodstuffs from undifferentiated sources (e.g. cellulose conversion, microbial chemosynthesis, algal biomass conversion) are all being studied to improve the caloric output per cultivated area. In addition, power efficiency (conversion of radiant or electrical energy to biomass) is being increased by improved lamp

design and solar energy collection systems.

Research, funded by NASA, regarding the production of food in a CELSS is well underway (Meissner and Modell, 1979). Human metabolic requirements have been well established and are the guiding element in the design of a food production system. However, requirements for micronutrients (vitamins, trace minerals), roughage, digestive system stimulators and limitation of harmful material intake are not as well defined (Tibbitts and Alford, 1982). Furthermore, psychological aspects involved with acceptance of vegetarian diets, organoleptic (taste, odor, texture etc.) qualities of processed foods and other unknowns are not yet understood, but these issues are all being addressed to some degree.

Food sources include a variety of species of higher plants (wheat, rice, white potatoes, sweet potatoes, soybeans, peanuts, lettuce and sugar beets) (Hoff, Howe and Mitchell, 1982) undifferentiated and synthesized food sources (Karel and Kamarei, 1984) and algae (Averner, Karel and Radmer, 1984b). Higher plants and algae in a CELSS food production system will use CO₂ and H₂O, both waste products of the crew, energy, and mineral nutrients (supplied by a waste processing system). In turn they meet the major nutritional requirements of the crew, supply O₂, and potable water. The growth characteristics of the plant species on waste processor supplied nutrients (Huffaker, Rains and Qualset, 1982) and

under a variety of environmental conditions (Raper, 1982) are being studied in order to increase productivity, optimize area and power requirements and further define waste processing needs.

The use of algae for food production is also being studied. The nutritive value of several algal species are well characterized (Karel, 1982). The extent to which algae will play a role in the food production subsystem will depend on many factors (Radmer et al, 1982; Radmer et al, 1984; Averner, Karel and Radmer, 1984b). One consideration is that algae may be required for atmosphere revitalization during periods of crop harvest or failure and that the biomass produced could serve as a reserve supply of food. Algal biomass may also serve as a primary source of protein as processing techniques improve.

Another approach to supplying crew nutritional requirements within power, mass and volume constraints involves techniques to maximize the caloric output of the photosynthetic organisms grown in space. One technique is the production of macronutrients from a limited range of food components (i.e. wheat, soybeans, algae, glycerol and cellulose). The food components supply one or more macronutrients to a food processing system which uses a variety of fabrication techniques to produce a wholesome and varied diet. Advantages of such a system are flexibility of raw material sources, improvement of the edible to nonedible biomass ratio (with concomitant

reduction in waste processor capacity requirements), ability to specify the exact nutritional value of foods produced and minimization of undesirable or toxic components in the diet. Problems may arise with the organoleptic characteristics of processed food and development or conversion of required technologies to the space environment (Karel and Kamarei, 1984).

Control Issues

Another important aspect of a CELSS will be the control mechanisms utilized. It will be critical to monitor, and when required, alter one or several system parameters to insure the uninterrupted availability of essential life support materials. The use of bioregenerative methods for life support complicates control strategy because of the complexity and unpredictable nature of organisms and ecosystems. Physical-chemical systems are more amenable to control due to their relative simplicity and known composition. NASA funded research is investigating ways to monitor and control the highly complex hybrid of biological and mechanical processes which will comprise a CELSS.

Control of a CELSS will exist on two distinct levels. Endogenous control occurs within organisms (e.g. biochemistry and genetics) and among groups of organisms and their environment (e.g. ecosystems). Implementation of endogenous controls will depend on the ability to manipulate biological processes for use as active control elements and not as passive elements upon which control must be exerted. Endogenous controls within a CELSS will reduce operational costs because once established they will require minimal maintenance.

Artificial or exogenous control will be required for all physical-chemical subsystems and in the environmental conditions to which organisms are exposed. There is a limit to the degree of artificial control which may be exerted on the biological components. Advances in control theory, genetic engineering and ecosystem management may increase the artificial control possible but a point will be reached where further artificial control is not possible or desirable. To achieve stability and resilience a CELSS will require a combination of exogenous human manipulations coupled to the endogenous response mechanisms of the organisms.

Many aspects of a CELSS will be poorly defined and contain significant degrees of uncertainty. It is the task of control theoreticians to devise techniques for creating a system that can accomodate a variety of perturbations and return the system to the original state.

Issues such as uncertain and poorly defined system control, resource allocation and control structures (Stahr et al, 1982; Auslander, Spear and Young, 1982; Auslander et al, 1984; Babcock, 1985) are important to control of a CELSS.

III. MATERIALS AND METHODS

Mouse Reactor

An inverted Pyrex 36.8 cm diameter Bell jar with a neoprene gasket is used to house a Nalgene metabolic mouse cage. A sheet of polycarbonate is securely attached to the Bell jar in order to achieve a gas-tight seal. Three gas-tight inlets allow electrical and gas connections to be made. Figure 8 shows the metabolic cage and associated equipment.

The metabolic cage allows measurement of food and water inputs and fecal and urinary output. Gases are input through a circular plenum at the bottom of the bell jar and circulated with a fan. The gas may be input from cylinders when mouse respiratory quotients are being measured or directly from the algal reactors when combined runs are being performed.

A computer-controlled solenoid valve is used as a safety device to prevent gas concentrations from exceeding standard physiologic ranges (i.e. below 18% O₂ or above 23% O₂ or 1.8% CO₂). An MKS Instruments pressure gauge is used to monitor internal pressure.

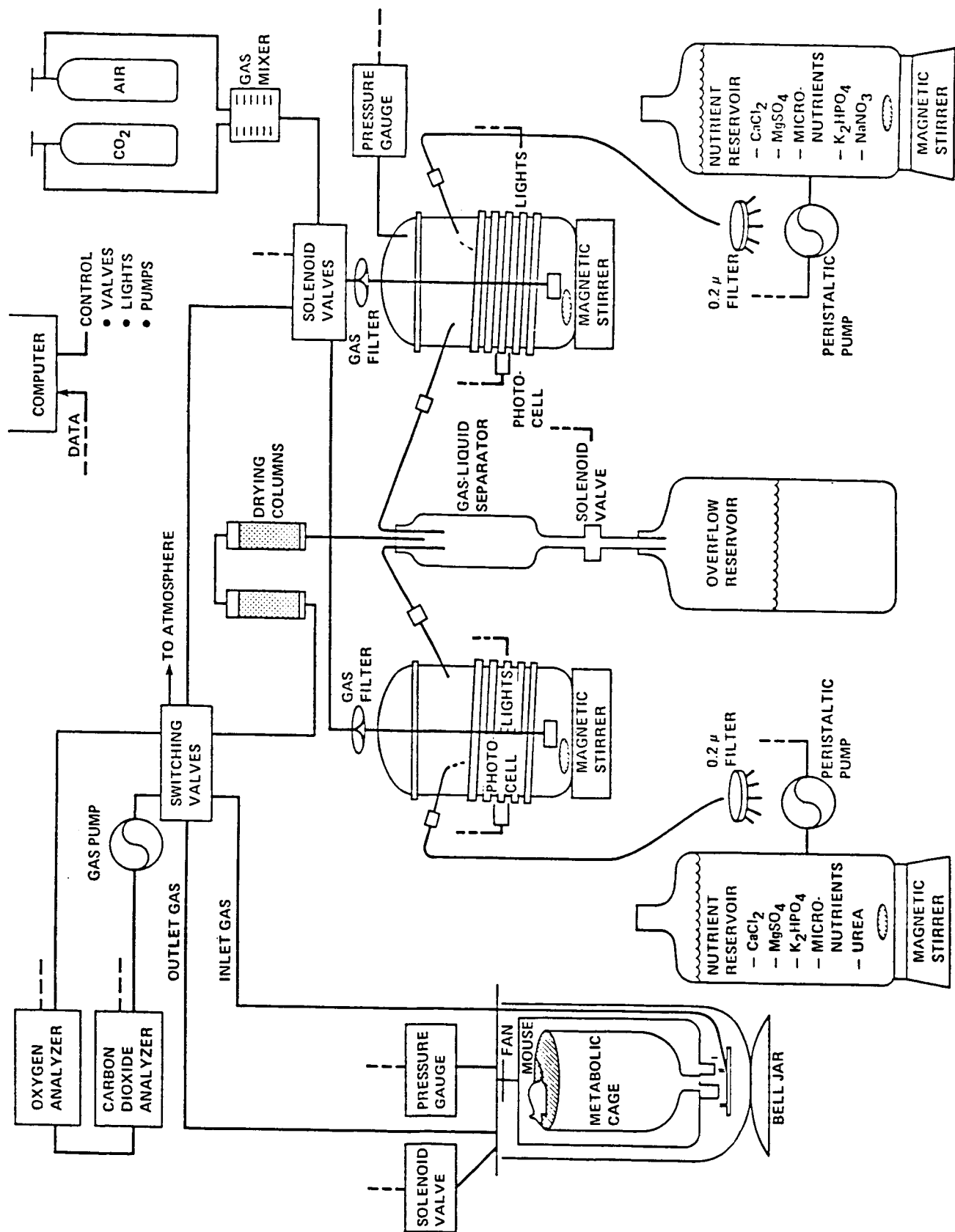


FIGURE 8. EXPERIMENTAL MOUSE-ALGAL SYSTEM

Algal Reactor

All experiments are conducted using either one or two Bellco water jacketed, double sidearm, one liter, spinner flasks. A neoprene rubber gasket is used between the ground glass top and bottom sections of the flask to obtain a gas tight seal. Each reactor is illuminated by five Sylvania, 40 watt, cool white circular fluorescent lamps. Light intensity at the reactor exterior reaches a maximum of 350 microeinsteins/m²/s. Control of light levels is done by turning lights on or off, either manually or via computer control.

The reactors are held at 36°C by use of a Haake water bath. Two percent CO₂ and compressed air are mixed in a Matheson gas mixer, filtered through a Matheson gas filter and a Millipore 0.2 micron gas filter and passed into solution with a glass-fritted sparger.

An MKS Instruments pressure gauge is used to monitor internal pressure and indicate the presence of gas leaks. Photocells are attached to the exterior of the reactors and allow for control of algal cell density by dilution with fresh media. The nutrient media composition can be seen in Figure 8. Nutrient media is autoclaved (121°C, for 80 minutes), held in a 12 liter Nalgene carboy and pumped via a peristaltic pump into the reactor. A Millipore 147 mm stainless steel filter holder with 0.2 micron cellulose acetate filters is used as a precaution

to prevent bacterial contaminants from entering the reactor.

As fresh media is pumped into the reactor to control cell density, overflow is collected in a gas-liquid separator. The reactor is run as a turbidostat using a photocell to detect light intensity at the reactor surface. Liquid flows into an overflow reservoir, the gas stream flows through two glass-wool filled tubes used to prevent bacteria from re-entering the reactor and a Drierite column is used to remove moisture prior to the gas streams entering the gas analyzers. A Lira infrared CO₂ analyzer (Model 303) continuously measures CO₂ concentrations between 0% and 2%. A Sybron/Taylor paramagnetic O₂ analyzer (Model 540A) continuously measures O₂ concentrations between 19% and 23%. Figure 8 diagrams the experimental mouse-algal system.

The reactors may be run in either flow-through mode or gas-closed modes. In flow-through mode, gas is sparged in from cylinders, passed through the gas analyzers and vented to the atmosphere. When measurements of algal assimilatory quotients, mouse respiratory quotients, or combined algal-mouse gas behavior are required the system is closed. Flow from the gas cylinders is stopped, a Metal Bellows Corp. air pump is turned on and valves are used to direct the gas stream from the analyzers back into the algal reactors. The gas stream may be diverted through the mouse reactor prior to entering the analyzer

loop for measurement of combined system behaviors.

Control System

The control system is based on a Dynabyte computer. Other control equipment includes the carbon dioxide and oxygen analyzers, pressure gauges, photocells, solenoid valves and several in-house built electronic components including sensor amplifiers for the photocells, a voltage regulator, an analog-to-digital signal convertor, and signal relays. A Soltec multi-pen strip chart recorder collects data. Data is also collected on the Dynabyte computer and transferred to a VAX 11/750 computer for permanent filing.

Software (BASIC) developed in-house allows a variety of controls to be exerted on the system. Lights and pumps may be turned on and off, valves may be opened or closed and optical density controlled to user-designated values. Thus, cell density and photosynthetic rate of each reactor may be controlled by either dilution of the reactor with fresh media or by increasing or decreasing the light intensity to the reactor. Reactor temperature is manually controlled with the water bath.

Wet-Air Oxidation

A Parr Instruments 316 stainless steel high pressure reactor (Model 4521) is used to oxidize mouse feces or algal cells. Water (200 ml) is added to a glass liner with a known mass of feces or algal cells. The liner is placed in the reactor which is then sealed and purged with O_2 (to remove atmospheric nitrogen) to a pressure of 400 psig. The reactor is heated to $275^\circ C$ for one hour, reaching a final pressure of 1500 psig. After cooling, the headspace in the reactor is analyzed for CO , CO_2 , O_2 and N_2 with a gas chromatograph (GC) built in-house. The GC consists of a column packed with Porapak N (100-120 mesh, Altech Associates), a sealed microdetector (Carle Instruments Inc.) and associated valves and recording equipment. After gas analysis the reactor is opened and the oxidate removed. The oxidate consists of liquid retained within the glass liner and liquid which recondenses between the liner and the stainless steel reactor wall. The oxidate is analyzed for total and kjeldahl nitrogen (NO_3^- and NH_4^+) and organic acids using standard wet-chemistry techniques. These samples are then stored separately at $4^\circ C$ for later analysis as algal nutrients.

Experimental Methods

A variety of photosynthetic gas exchange experiments have been conducted using the system. Instruments measure and record the changes in CO₂ and O₂ within the system. Algal growth conditions (light intensity and cell density) are varied to control the photosynthetic rate of the cultures. Variation of nitrogen species (urea and nitrate) alters the algal assimilatory quotient (AQ) and allows control of the CO₂ consumption/O₂ production ratio. Combinations of rate and ratio manipulations are used to maintain atmospheric levels of CO₂ and O₂ at physiological levels.

Preliminary work determined the assimilatory quotient of Chlorella pyrenoidosa grown on nitrate and urea and under different light intensities and optical densities. Early combined mouse-algal experiments indicated that two algal reactors would be required to supply the O₂ requirements of the dwarf mouse. Combined mouse-algal runs have been conducted and demonstrate that stable conditions can be maintained for periods up to several hours. Respiratory quotients of the mouse have been measured for short time periods (hours) which include awake, sleeping and eating periods.

Progress in quantifying the rates of CO_2 and O_2 exchange have been hampered by the presence of bacteria in the algal cultures. The bacteria present are contaminants and are not intended to represent the decomposer (waste processing) functions of a CELSS. The effect of bacterial respiration on gas exchange dynamics within the system is unclear and work continues to quantify what that contribution is. Preliminary data indicates that there is a decrease in the apparent algal AQ between axenic and bacterially contaminated cultures. The ability to eliminate bacterial populations from the system is a limiting factor both in the experimental system and in a CELSS. Bacterial populations are monitored by the aseptic removal of samples from the algal reactors. Samples are plated on standard trypticase soy agar (TSA) plates and incubated for 48 hours at 30 °C. Plate counts are done for both bacteria and algae by use of the standard spread plate method using TSA plates.

In conjunction with the photosynthetic gas exchange experiments a set of experiments exploring mass closure have been initiated. Waste products (feces and harvested algal cells) are abiotically oxidized and used as an algal nutrient supply. Quantification of material flows through the mouse reactor are also conducted. Figure 6 diagrams the experimental mouse-algal system with waste oxidation included.

One set of element recycle experiments involves the use of system waste products for growth of algae. Fecal material or harvested algal cells are collected, lyophilized and placed into the wet-oxidation reactor. Water or spent algal growth media and oxygen are added and the temperature is raised to 275 °C for one hour. After cooling the wet-oxidate is removed and added to algal growth flasks. Dilutions of the oxidate are made and the flasks are autoclaved and then inoculated with Chlorella pyrenoidosa.

Growth experiments using the effluent from the WAO are conducted in flasks using CO₂ enriched air. The algal reactors are not yet supplied with nutrients derived from waste materials. Attributes of the oxidized material as a nutrient source are made by comparing the optical density (420 nm) of test flasks with control flasks grown on the standard media. Measurements of optical density are made over a period of several days. Urine will be filtered and used directly as a nutrient source without intermediate oxidation. The inputs to (O₂, H₂O and wastes) and outputs from (chiefly CO₂, N₂ and H₂O) the waste processor are measured and used in determining the system's mass balance.

A second set of material recycle experiments quantifies the flow of food, water, feces and urine through the mouse reactor. A commercially available metabolic cage is used to determine the mouse element balance. Elemental analysis of the mouse food, feces and urine have been conducted using a Perkin Elmer (Model 240B) elemental analyzer. Percentage of carbon, hydrogen and nitrogen are obtained directly while percent oxygen is calculated by subtracting ash weight and carbon, hydrogen, nitrogen values from total sample weight. The contribution of other elements (phosphorus, sulfur) is assumed to be negligible.

IV. DATA ANALYSIS AND DISCUSSION

Atmosphere Stabilization

Respiratory Quotients

Figure 9 shows the changes in O_2 and CO_2 concentrations over time in a gas-closed system containing a mouse only. As expected, the mouse consumes O_2 and exhales CO_2 . The rates of CO_2 production and O_2 consumption are determined from the slopes of these lines, the CO_2/O_2 ratio is the RQ. RQ's of the dwarf mouse within the system have been determined to be 0.975 ± 0.06 (sample size (n) =5). Mouse exhalations result in an unmeasured quantity of water vapor which must be accounted for if complete mass balance is to be determined.

Assimilatory Quotients

Figure 10 depicts the O_2 and CO_2 atmospheric concentrations over time in the gas-closed algal reactor grown on nitrate at an optical density of 0.61 and a light intensity of 350 microeinsteins/ m^2/s . CO_2 is consumed in the photosynthetic reaction yielding O_2 . Rates of CO_2 consumption and O_2 production are calculated from the slopes of the lines, the CO_2/O_2 ratio is the AQ. The opposite effect is seen from Figure 9 and it becomes obvious that either algae or a mouse alone in the

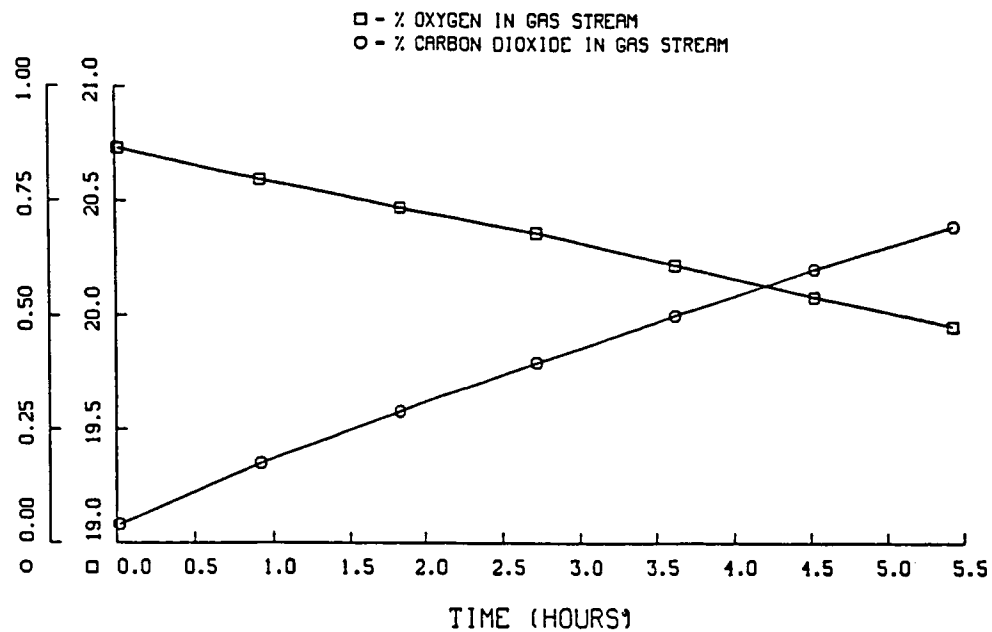


FIGURE 9. CO₂-O₂ RELATIONSHIP IN A GAS-CLOSED MOUSE SYSTEM
Measurement of Mouse Respiratory Quotients

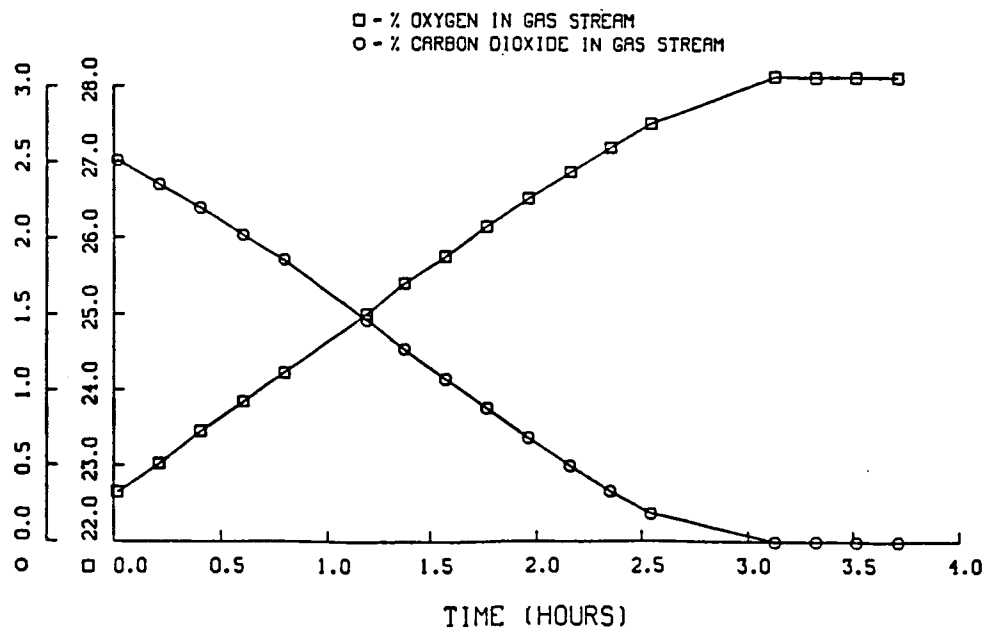


FIGURE 10. CO₂-O₂ RELATIONSHIP IN A GAS-CLOSED ALGAL SYSTEM
Measurement of Algal Assimilatory Quotients, Nitrate (OD 0.61)

gas-closed system will deplete one atmospheric component and increase the other. Therefore, in order to maintain O_2 and CO_2 within specified physiologic limits it is essential to couple the algae (photoautotroph) and mouse (heterotroph) within a single atmosphere. Measurement of algal AQ's has been accomplished under a variety of operating conditions.

The maintenance of axenic algal cultures (i.e. a pure algal culture as determined by absence of bacterial colonies on TSA plates) for extended periods of time has proven to be difficult. In a functional space-based CELSS it would seem that such a task will be even more difficult. Therefore, determination of the variation of AQ between axenic and bacterially contaminated cultures has been made. The presence of bacteria in the algal cultures create a variety of control problems within the algal reactors. Competition for nutrients (especially nitrogen) can cause marked inhibition of algal growth. Determination of AQ is complicated by varying levels of bacterial contamination. Operation of the reactors to prevent contamination requires extensive preventive measures.

Figure 11 illustrates the difference in AQ between axenic and contaminated algal cultures. AQ's of 0.50 ± 0.07 ($n=25$) and 0.67 ± 0.02 ($n=5$) have been observed for contaminated and axenic cultures respectively. It is probable that in axenic cultures the apparent AQ is higher

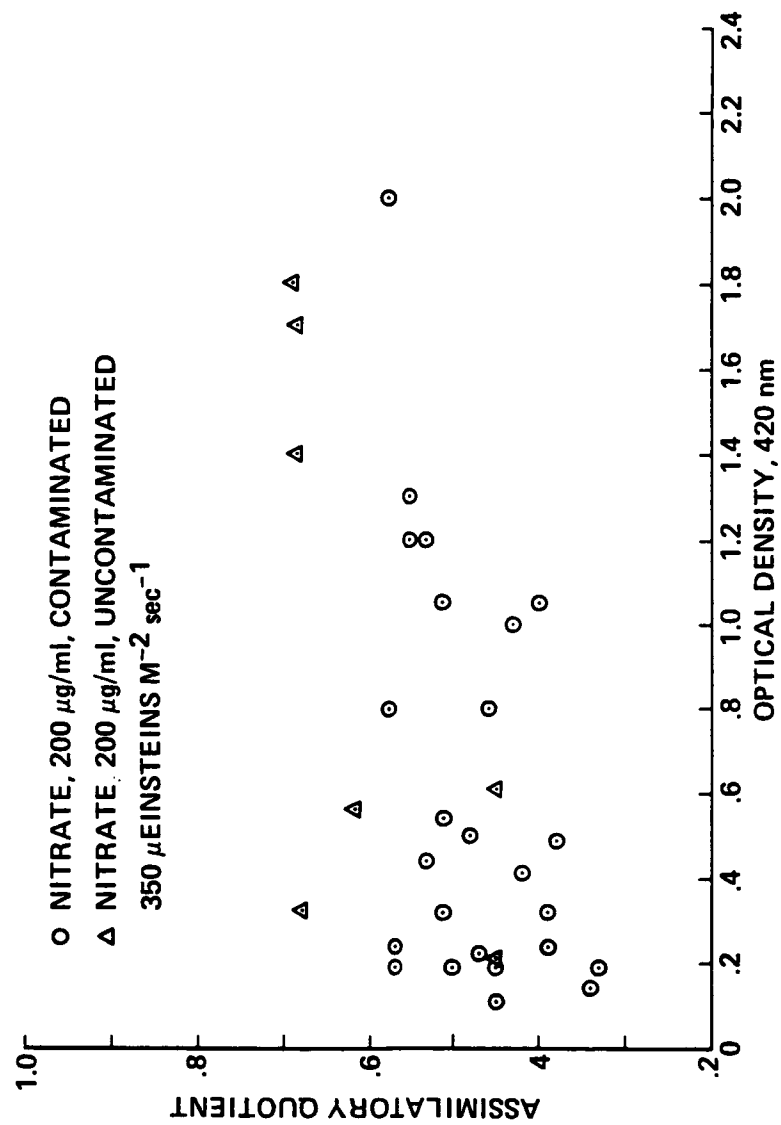


FIGURE 11 VARIATION OF ASSIMILATORY QUOTIENT AS A FUNCTION OF OPTICAL DENSITY

because there is no bacterial respiration consuming O_2 . The small standard deviation (SD) of 0.02 for axenic values is in contrast to the larger SD (0.07) of contaminated AQ values. This is probably due to the relative sizes of the bacterial populations in different cultures. A heavily contaminated algal culture should have a lower apparent AQ because the bacterial respiration will be greater. Thus variations in AQ seem to be dependant on the degree of bacterial contamination. The algal populations are on the order of 2×10^7 cells/ml while the bacterial population is around 1×10^4 cells/ml.

The effects seem to be significant even with small bacterial populations. More precise determinations of relative population sizes are required in order to verify and quantify the effect of bacterial respiration on the measurement of algal AQ. The two uncontaminated points with AQ's less than 0.5 (Fig. 11) were obtained from a culture which initially showed no bacteria but eventually became contaminated. The five points near an AQ of 0.7 were from a culture which remained axenic. Perhaps a low, undetectable bacterial population was present in the former case indicating that low level contamination can effect the AQ. Successful operation of an algal photosynthetic gas exchanger will require monitoring and control of bacterial populations and a significant data base concerning their impact on system functions. Also,

the low correlation coefficient (r) for both axenic ($r=0.42$) and contaminated ($r=0.37$) indicate that the AQ is relatively independent of the optical density. Thus AQ variation between axenic and contaminated cultures is most likely due to bacterial respiration altering the apparent AQ.

Manipulation of the algal AQ has been studied by varying the nitrogen source supplied in the algal growth media. Algal cells grown under the same light intensities with either nitrate or urea have AQ's of 0.50 ± 0.07 ($n=25$) and 0.77 ± 0.12 ($n=28$), respectively. Differences in biosynthetic pathways result in different CO_2 consumption and O_2 production. This variation is being examined further in order to establish the relationship between nitrogen source and AQ. Such information will be useful for designing endogenous control strategies based on the inherent responses of algal cultures to variation in nitrogen source. For example, multiple reactors with different AQ's may be desirable and the use of different nitrogen sources to maintain the required AQ's may be an effective way to achieve this goal. Waste streams and waste processor outputs will contain different nitrogen species which could be used to maintain algal cultures with desired AQ's.

Combined Algal-Mouse Experiments

Figure 12 illustrates atmosphere behavior in combined algal-mouse reactors in a condition where the mouse respiration is greater than the algal photosynthesis. R1 and R2 designate the two algal reactors with their respective OD's. At high optical densities ($OD=7.5$) the algae are not consuming as much CO_2 as the mouse exhales. Similarly, the O_2 consumption of the mouse exceeds the O_2 production of the algae. The high OD and apparent high photosynthetic rate (PR) would seem to indicate a system state where photosynthesis would exceed respiration. However, the light use efficiency at such high OD's is decreased by shadowing effects. Therefore the CO_2 utilization efficiency is not as great and the photosynthetic contribution to the system is correspondingly less. Increasing the light intensity at 1.1 hours does not change system dynamics enough to equalize the photosynthetic contribution to the respiratory contribution. Side effects such as auto-toxification, nutrient competition, clumping and foaming can also limit the photosynthetic output of high OD algal cultures.

Figure 13 exhibits a similar system state (i.e. CO₂ increasing, O₂ decreasing) at lower optical densities (OD 0.345) and with a different nitrogen source (urea). Low OD's, even with high light intensities, cannot generate sufficient photosynthetic capacity to equalize the mouse respiration. Both Figures 12 and 13 depict a situation in which the system state is characterized by increasing [CO₂] and decreasing [O₂]. This system state is referred to as being in a respiratory mode.

Photosynthetic mode occurs when [CO₂] is decreasing and [O₂] is increasing. Figure 14 depicts both system modes, photosynthetic and respiratory, occurring within the same run. Initially photosynthesis is greater than respiration at both 700 microeinsteins/m²/s and 460 microeinsteins/m²/s (during the first two hours). As more lights are turned off (170 microeinsteins/m²/s during the third hour) the PR of the cultures decreases and the system state switches to where respiration is greater than photosynthesis. As lights are turned on (during the fourth and fifth hours) the system returns to a photosynthetic mode. Figure 14 demonstrates that the algal PR can be controlled by variation of light intensity and hence effect the relative atmospheric gas concentrations within the system. This is mostly an exogenous control strategy because it relies upon manipulation of non-biological factors (lights). The

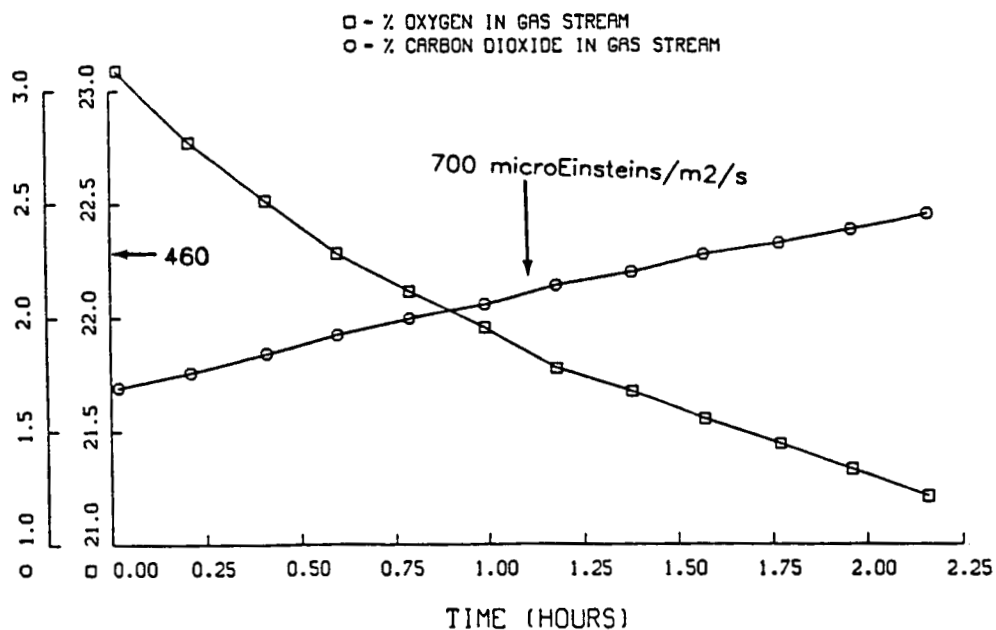


FIGURE 12. CO₂-O₂ RELATIONSHIP IN A GAS-CLOSED ALGAL/MOUSE SYSTEM
Effects of Variation in Light Intensity (Nitrate R1 00 7.4, R2 00 7.6)

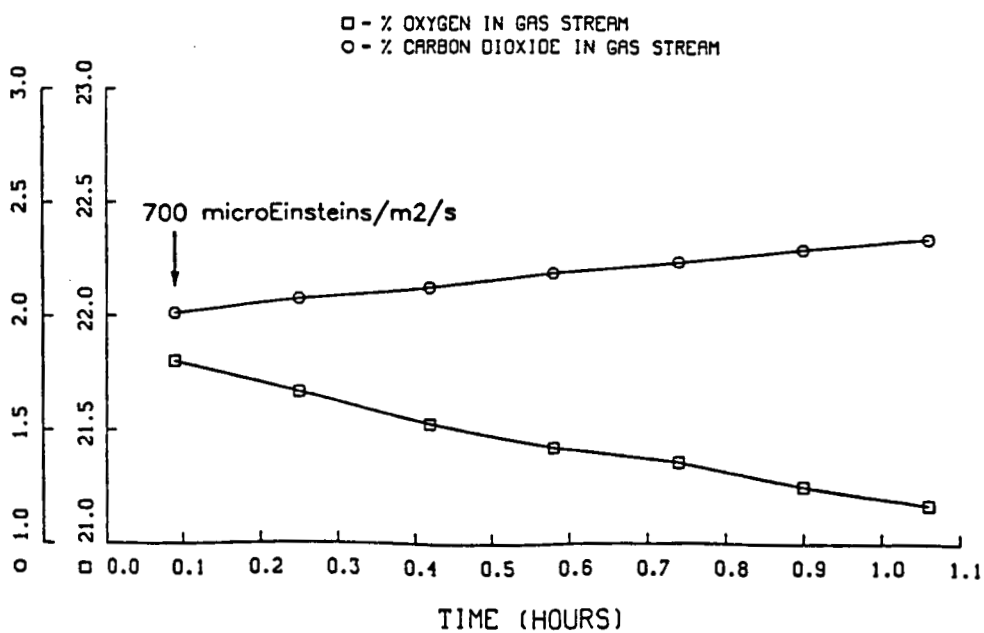


FIGURE 13. CO₂-O₂ RELATIONSHIP IN A GAS-CLOSED ALGAL/MOUSE SYSTEM
Effects of Variation in Light Intensity (Urea R1 00 0.36 R2 00 0.33)

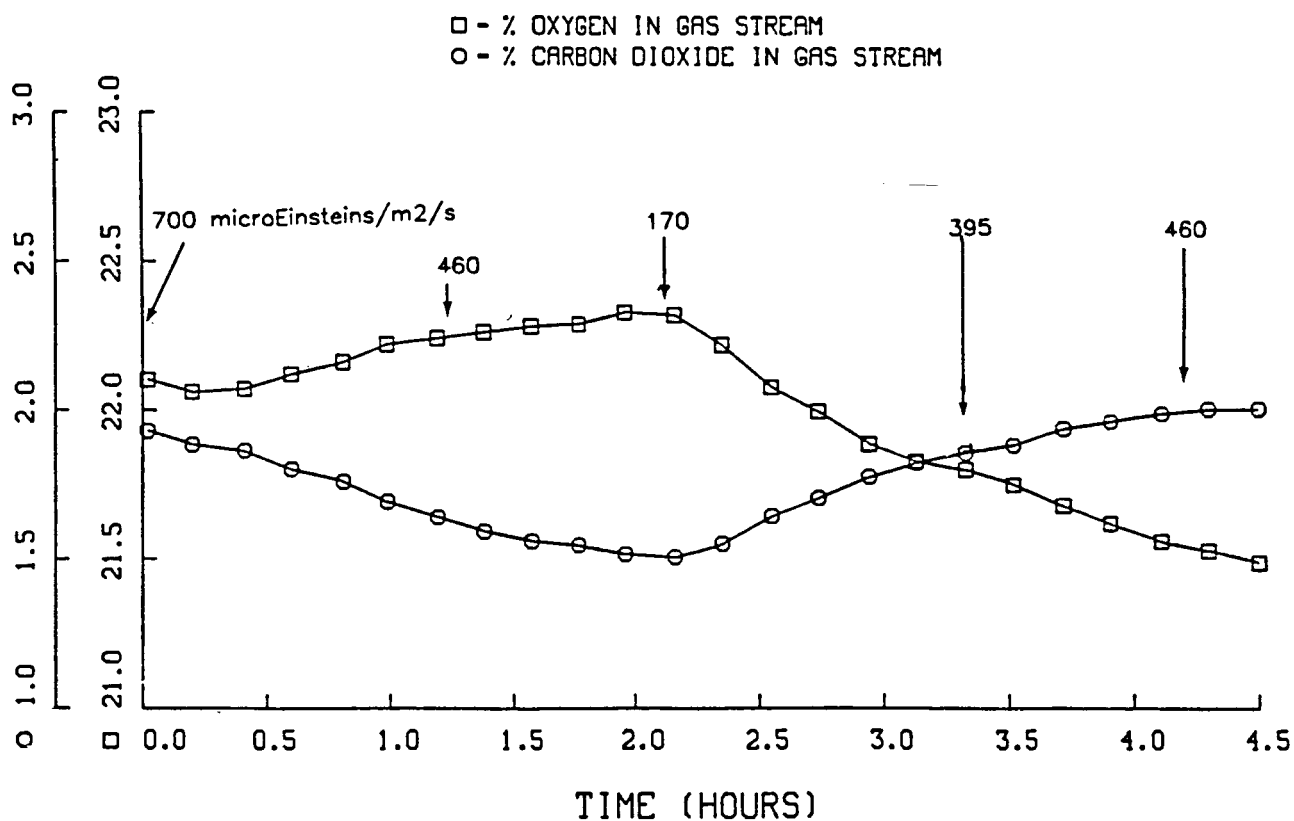


FIGURE 14. CO₂-O₂ RELATIONSHIP IN A GAS-CLOSED ALGAL-MOUSE SYSTEM
Effect of Variation in Light Intensity (Nitrate R1 OD 2.1 R2 OD 1.7)

response of the algal cells to light intensity is, of course, an endogenous response. Variation of light intensity apparently does not affect the ratio of CO₂ consumption to O₂ production (AQ) except at very low light intensities when algal photosynthesis approaches algal cellular respiration. Further experimental data will be necessary to determine if low light intensities can be used as to manipulate AQ or if light intensity variation will only be useful as a photosynthetic rate manipulator.

Short-term steady state concentrations of O₂ and CO₂ have been demonstrated using either NO₃⁻ only (Fig.15) or urea only (Fig.16) in both reactors. At moderate light intensities (460 microeinsteins/m²/s) and at a combined OD approaching one (OD=0.88), an algal system growing on nitrate exhibits a photosynthetic trend. Slightly reducing the light intensity shifts the system to a point where photosynthesis nearly equals respiration. The imbalances in the system will, in time, lead to unacceptable variation in [CO₂] and [O₂] unless light levels are monitored and adjusted to meet changing atmospheric concentrations.

An algal system growing on urea at a combined OD on near one (OD=0.93) and an initial light intensity of 460 microeinsteins/m²/s shows a respiratory trend (Fig. 16). Increasing light to 570 microeinsteins/m²/s slows the respiratory trend and at 700 microeinsteins/m²/s the

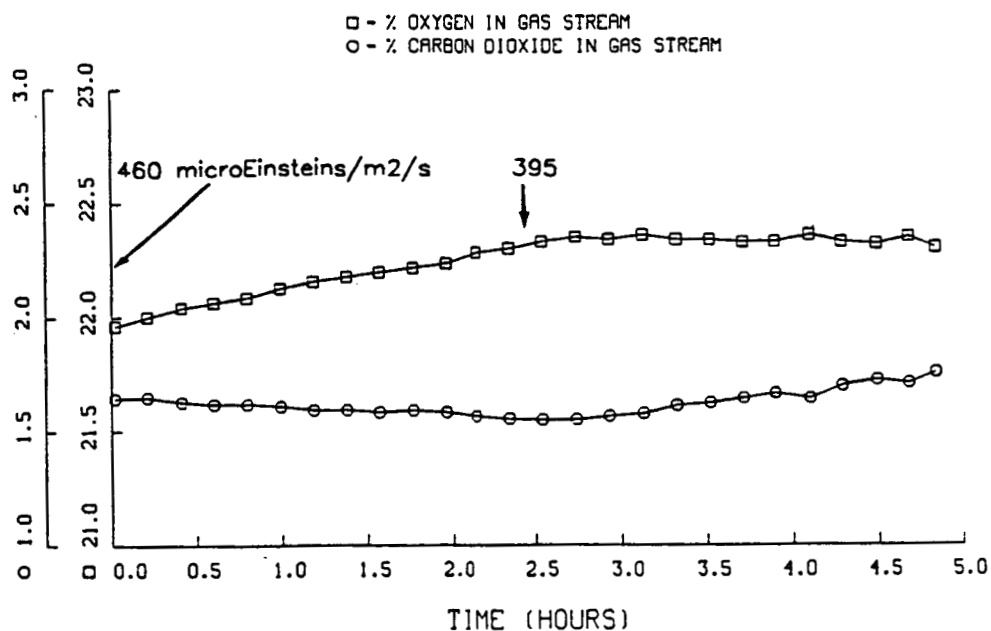


FIGURE 15. CO₂-O₂ RELATIONSHIP IN A GAS-CLOSED ALGAL-MOUSE SYSTEM
Effect of Variation in Light Intensity (Nitrate R1 00 0.95 R2 00 0.80)

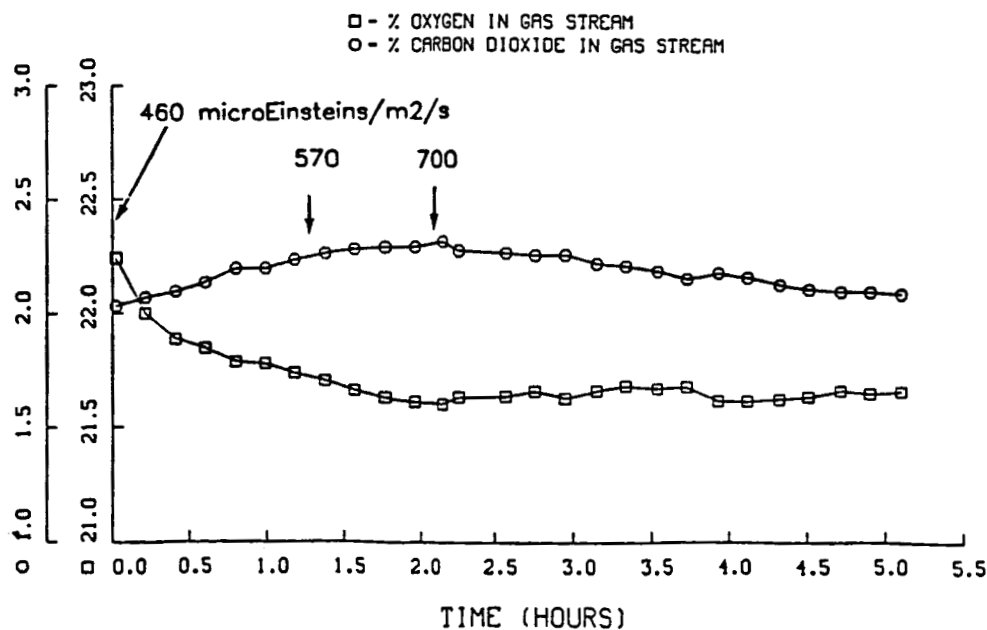


FIGURE 16. CO₂-O₂ RELATIONSHIP IN A GAS-CLOSED ALGAL-MOUSE SYSTEM
Effect of Variation in Light Intensity (Urea R1 00 1.0 R2 00 0.85)

trend reverses to a photosynthetic mode. More precise control of lighting would allow maintenance of stable $[CO_2]$ and $[O_2]$, at the cost of intensive system monitoring and control.

Figures 12 and 13 demonstrate that system conditions exist where light intensity variations cannot overcome either too high or too low optical densities or the respiratory contributions to system dynamics. Figures 15 and 16 show that conditions also exist where light intensity variations can sufficiently alter system dynamics and allow control of the mismatch between AQ and RQ. Whether the responses seen are due to changes in AQ or simply from photosynthetic rate changes is not yet determined.

Reliable system operation has also been achieved using two reactors each growing on different nitrogen sources and each demonstrating a characteristic AQ (Fig. 17). By using reactors with different AQ's it is possible to balance the photosynthetic contribution of the system to the respiratory contribution. Figure 17 exhibits the longest gas-stable system response although it does not demonstrate a perfect match of AQ to RQ. The use of nitrate and urea reactors together seems to allow greater control although further experiments are required to fully evaluate system responses. A further advantage of the system state seen in Figure 17 is that control is achieved without having to vary light intensity. Thus a single

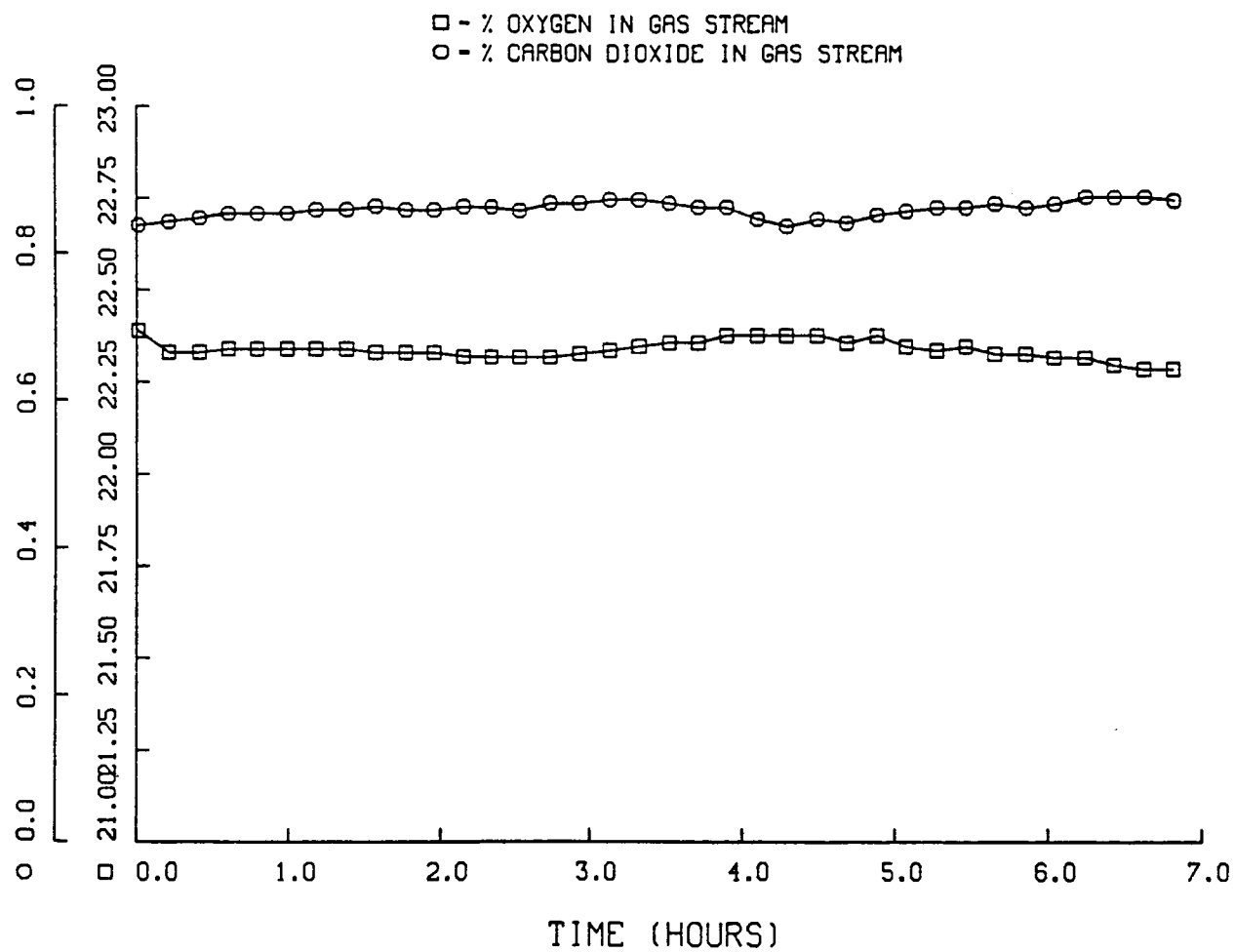


FIGURE 17. CO₂-O₂ RELATIONSHIP IN A GAS-CLOSED ALGAL-MOUSE SYSTEM
 R1 Nitrate (OD 1.64): R2 Urea (OD 4.00): 700 microEinsteins/m²/s

factor (nitrogen source) yields stability, thereby reducing the control requirements.

Endogenous control based on manipulation of the AQ ratio is more precise than rate control because it allows a closer match of AQ to RQ. If AQ and RQ values within the system are exactly equal there will be a perfect balance of CO_2 and O_2 within the system, excluding the tie up of carbon and oxygen in biomass. However, as the difference in AQ and RQ increases more intensive control measures (such as variation of light intensity) will be required in order to maintain steady-state concentrations of CO_2 and O_2 . The goal of future experiments will be to bracket the mouse RQ with algal reactors exhibiting different AQ's. The implementation of endogenous control of AQ will reduce the requirement for exogenous controls thereby minimizing the variation of atmospheric $[\text{CO}_2]$ and $[\text{O}_2]$. By minimizing the AQ/RQ mismatch the need for more expensive (i.e. power, labor, equipment) controls is reduced.

Figures 18 and 19 were derived from experimental data collected with the algal system operating under varying regimes of light intensity and optical density. As the slopes of O_2 and CO_2 changed from positive to negative, the system mode would change. The point or area where this change occurs is shown in Figures 18 and 19. These figures demonstrate that there is a "cross-over area" for nitrate and urea grown cultures where the

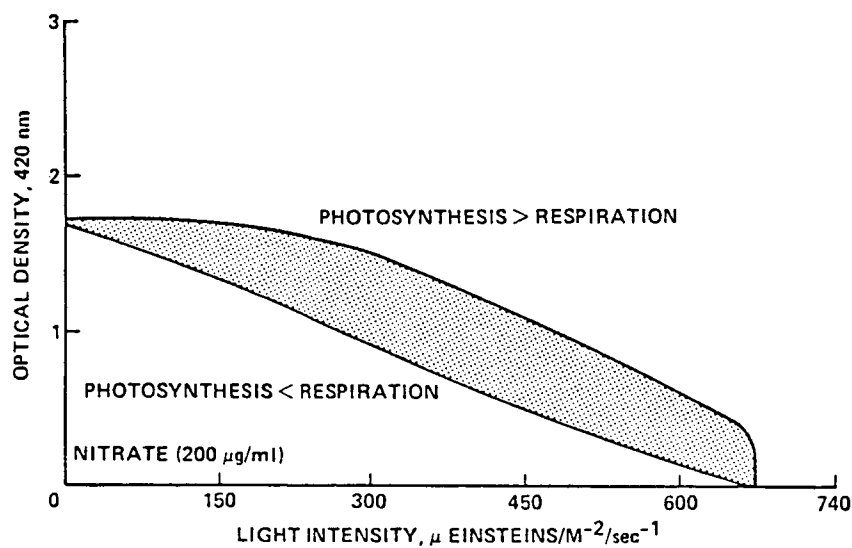


FIGURE 18. CROSSOVER AREA AS A FUNCTION OF OPTICAL DENSITY AND LIGHT INTENSITY

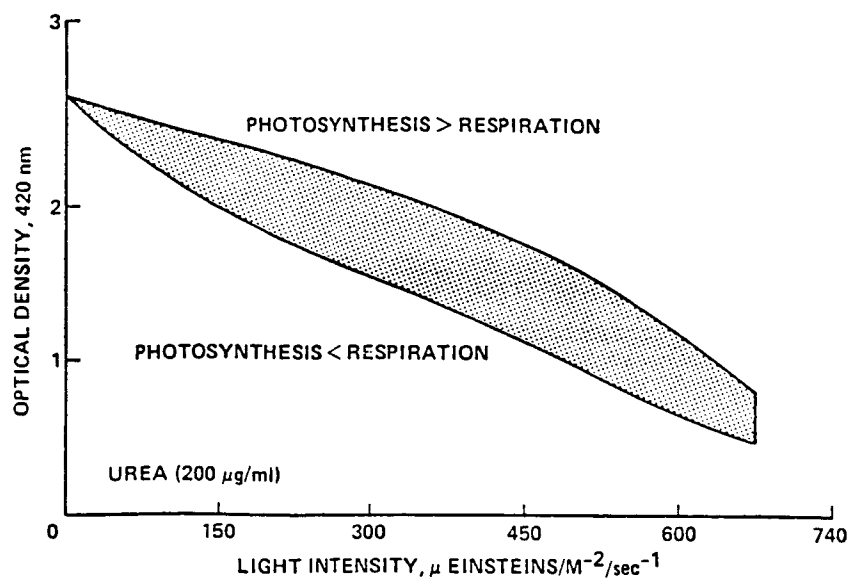


FIGURE 19. CROSSOVER AREA AS A FUNCTION OF OPTICAL DENSITY AND LIGHT INTENSITY

optical density and light intensity will determine whether the system is in a photosynthetic or respiratory mode. This crossover area is different for urea and nitrate making it possible to determine a variety of culture conditions which might bracket the RQ and allow control of gas exchange with minimal manipulation.

The data from Figure 17 do not correlate with the data from Figures 18 and 19. The predicted system state given the light intensities and optical densities of the two cultures shown in Figure 17 would indicate an overall system mode as photosynthetic. Thus one would expect to see a trend towards CO₂ depletion and O₂ buildup. However, the experimental data of Figure 17 indicate a respiratory trend developing after 7 hours. There are two possible explanations for this discrepancy. First and most obvious is that the crossover areas contain significant uncertainty. More data is required to refine the crossover area, which will allow more accurate prediction of system behavior. Second, it is not clear that the system will behave as predicted by subsystem behavior. Urea and nitrate cultures growing alone may exhibit different responses than combined urea/nitrate cultures. The sum of the parts may not in fact equal the sum of the whole. A urea/nitrate crossover area may need to be developed in order to accurately predict the response of that system to variations in light intensity and optical density.

These crossover area curves demonstrate that manipulation of the algal photosynthetic rate can allow significant degrees of control of the system. Variations in both light intensity and optical density will shift the rates of photosynthesis, however it has not been determined if these variations shift the ratio of CO₂ consumption to O₂ production (AQ). The shifts between photosynthetic and respiratory modes may in fact be due to a shift in both rates and ratios of gas production and consumption.

Experiments conducted at different light levels do not indicate a significant difference between AQ, urea grown cultures at 350 microeinsteins/m²/s of light exhibited and AQ of 0.77 ± 0.12 while urea cultures grown at 230 microeinsteins/m²/s had an AQ of 0.59 ± 0.05 . Bacterial contamination effects were not accounted for and repetition of these experiments is required to confirm or deny the hypothesis that AQ does not vary with light intensity.

It is likely that bacterial respiration is occurring in the mouse reactor. Although feces and urine are separated, thereby lowering the water content of the feces, bacterial decomposition of the feces will still occur. Contamination of the urine will probably also occur leading to even more bacterial activity. The contribution to atmospheric dynamics by bacterial contamination within the mouse reactor has not been measured. The size of

bacterial populations will vary within and between runs. Future experiments must determine the impact of bacterial decomposition of mouse waste products to system dynamics.

Precautions to prevent bacterial contamination of feces, urine and the algal reactors will improve the ability to accurately assess system dynamics. Operation of axenic algal runs has been accomplished by using a variety of techniques. Separation of feces and urine and the flow of gas through the fecal storage helps to retard bacterial growth. Baseline measurements of bacterial respiration in a gas-closed system without a mouse need to be done in order to separate bacterial respiration from mouse respiration.

Mass Balance

A significant degree of atmospheric stabilization appears to have been achieved within the gas-closed mouse-algal system. The short-term demonstration of CO₂ and O₂ balance within the system indicates that control may be exerted on the photoautotrophic (algal) component in order to maintain both [CO₂] and [O₂] within acceptable physiologic ranges. However, to determine the long-term balance of elemental flows within the system quantification of the bio-elemental flows through system compartments is required. Carbon and oxygen are held in materials such as living tissue, water, urine and nutrients within the system and thus will move at different rates and at different concentrations than would be observed if only CO₂ and O₂ are observed.

Measurement of the elemental flows within the system is necessary to develop an accurate view of system flow dynamics. The system elemental flows (carbon, oxygen, nitrogen, hydrogen (C,O,N & H)) are quantified as they pass between three compartments: mouse, algal and waste processor (Fig. 20). Elemental analysis of mouse food, feces, urine and algal cells has been conducted using an elemental analyzer. Known elemental composition of algal media, waste processor inputs and measured waste processor outputs are also used to calculate mass balances through the compartments.

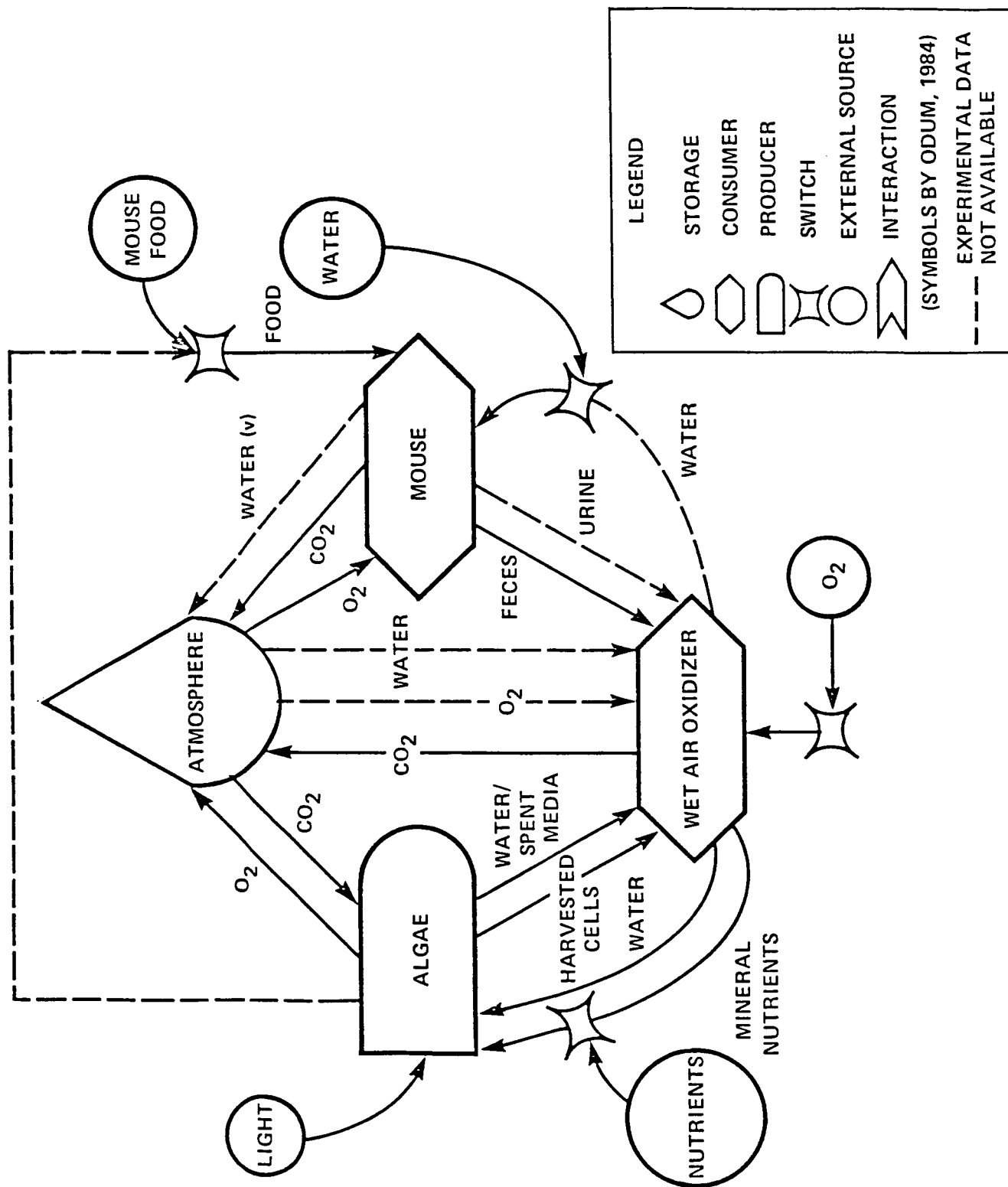


FIGURE 20. SIMULATION MODEL; MOUSE/ALGAL/WAO SYSTEM

A variety of assumptions and limitations must be made within this approach. In order to demonstrate mass closure, the algal cells must be used as the source of mouse food although this has not been done experimentally. Given the known composition of each stream (mouse food and algal cells), we can investigate the assumption that the algal biomass produced could replace the mouse chow. However, the nutritional quality of algal cells and the response of mouse metabolism to the algal diet is not established. Also, the composition of spent algal media is not directly measured, instead it may be calculated from the known initial composition minus the measured algal cell uptake values. The lack of experimental data in this area precludes exploration of these relationships in this thesis.

To analyze mass flow through the mouse-algal-wet oxidizer system a simulation model has been developed, an adaptation of a related mass flow model based on humans, wheat and a WAO. The development of the simulation model has enhanced the analysis of experimental data and will be especially useful for predicting the behavior of the experimental system by varying conditions within the model. Initially the experimental data will be used to validate the model. Once validated, the model can be used in place of the experimental system to more rapidly determine the flow of mass under a variety of operating conditions. The basic structure of the model (Fig. 20) is

explained in detail below.

An important consideration in the development of the model has been the degree of complexity incorporated into it. There are several levels of complexity which have been considered. These include the compartment level (i.e. mouse and its environmental conditions), the organism level, the organ system level, the cellular level, the biomolecular level and the atomic level. Increasing the detail of the model to include the atomic interactions associated with biochemical reactions is considered to be too detailed. Similarly the compartment level alone is too general to yield useful information on the flow of materials through the system. It has been determined that the compartment level containing the biomolecular level as a subsystem is most appropriate. The important biomolecules that will be incorporated are carbohydrates, proteins and lipids. Other than accounting for C,O,N & H in such biomolecules there may be no need to distinguish these compounds further in terms of detailed biochemical pathways. In fact, some areas of the model may not include detail at this level. For example, for certain segments of the model it may be necessary to include only the living component as a whole and not to break it down into specific molecules.

Model structure may be best shown by tracing the flow of one element, such as carbon. The model has been broken into compartment segments to clarify the interactions occurring within each. Figure 21 shows the flow of carbon through the mouse compartment. Carbon may introduced into the model from either mouse food or algal cells in the forms of carbohydrate, lipid and protein. Once eaten by the mouse, the metabolism of carbohydrate and lipid yields energy by conversion of simple sugars to CO_2 and H_2O in the presence of O_2 . Additionally, the fats and sugars can be stored by the mouse. The carbon in proteins is used in maintenance of the mouse, the net flow may be set to zero or to whatever value experimental data suggests. Thus carbon introduced to the mouse is 1) metabolized to CO_2 and exhaled, 2) excreted in feces and urine or 3) retained in the mouse.

Table 1 shows the mouse mass balance which has been calculated manually from information contained within the model and from experimental data. This data will be used to validate the mouse portion of the simualtion model. The data was collected in two ways; values for CO_2 consumption and O_2 production were calculated from the slopes of RQ measurements made within the gas-closed mouse system, values for food and water consumption and fecal and urine production were obtained from metabolic cage experiments conducted outside the bell jar. The value for water vapor exhalations was not measured and thus was

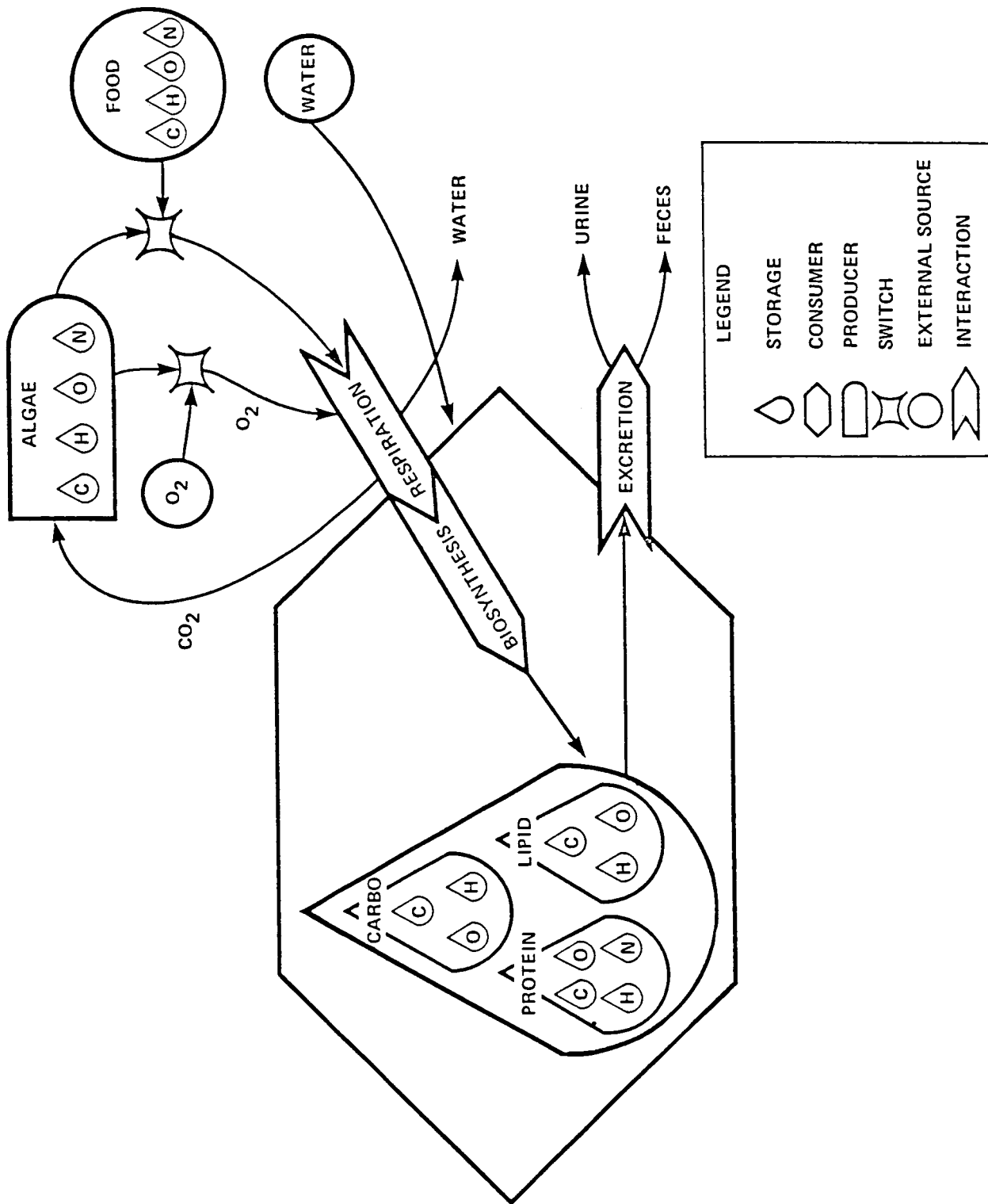


FIGURE 21. SIMULATION MODEL; MOUSE COMPARTMENT

TABLE 1: MASS BALANCE OF A DWARF MOUSE

(All values in grams/mouse/day)

	<u>TOTAL</u>	<u>C</u>	<u>H</u>	<u>O</u>	<u>N</u>
<u>OUT</u>					
Urine Solids	0.1319	0.0267	0.0070	0.0250	0.0357
Urine Water	0.7300	-	0.0803	0.6497	-
Fecal Solids	0.1911	0.0684	0.0101	0.0591	0.0088
Fecal Water	0.3549	-	0.0390	0.3159	-
Water Exhaled	2.162	-	0.2393	1.914	-
Carbon Dioxide Exhaled	2.19	0.597	-	1.592	-
Subtotals	5.7671	0.6921	0.3757	4.556	0.0445
Storage (in mouse)	0.1260	0.0306	0.0133	0.079	0.0032
Total Out	5.8931	0.7227	0.3890	4.635	0.0477
<u>IN</u>					
Food	1.684	0.7447	0.1031	0.6399	0.0674
Water	2.680	-	0.2948	2.385	-
Oxygen Consumption	1.610	-	-	1.610	-
Total In	5.974	0.7447	0.3979	4.6349	0.0674
Difference	0.081	0.022	0.0089	0.0	0.0197
% Recovery	98.65	97.05	97.8	100.0	70.8

calculated from the O, H values remaining after all other inputs and outputs were balanced. The ratio of O to H was very close to the expected 8:1 ratio and the assumption was made that this remaining mass must be the water vapor content.

The high percent recovery seen in Table 1 indicates that the mouse system demonstrates closure for the primary bioelements. Carbon and hydrogen flows show a 97% recovery, the 100% recovery of oxygen is found because the excess oxygen was all placed in the water vapor column and a stoichiometric amount of hydrogen was added to the hydrogen value. The 71% recovery of nitrogen is lower than would be expected. Possible sources of loss include the bacterial denitrification of urea in urine and feces to N_2 , the loss of urine in the collection process by either volatilization or adherence to surfaces and the variation of solids contents in both urine and feces between different experimental runs.

Returning to our trace of carbon through the system; the CO_2 exhaled by the mouse is introduced to the algae as its sole source of carbon (Fig. 22). Algal photosynthesis then converts the CO_2 into simple sugars and O_2 in the presence of water and light. Biosynthesis of the sugars to lipids and proteins may be incorporated into the model, or the model can be run using only an algal biomass pool in place of the more specific biomolecules. Theoretically the algal biomass may then be

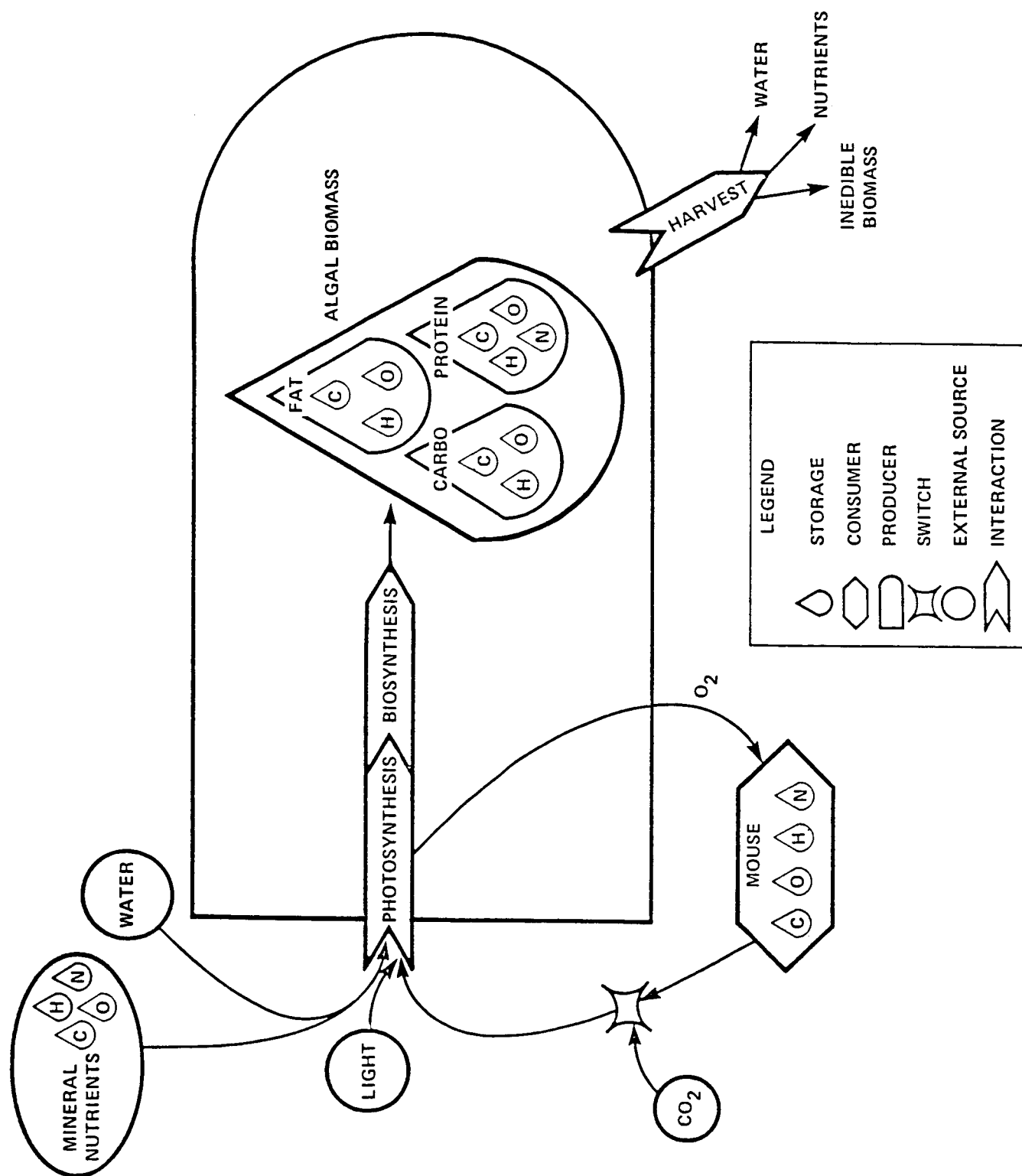


FIGURE 22. SIMULATION MODEL; ALGAL COMPARTMENT

recovered for use as a mouse food. Waste algal biomass may also be directed to the waste oxidizer.

The waste oxidizer receives carbon from the mouse feces and oxidizes it into CO_2 , low molecular weight organic acids (primarily acetic) and CO in the presence of O_2 supplied externally or from the algal compartment (Fig. 23). When the carbon from mouse feces has been oxidized to CO_2 and this CO_2 is then metabolized to algal biomass the cycle of carbon through the system is complete. Similar paths may be traced for oxygen, nitrogen and hydrogen so that an overall system balance for the major biomolecules may be observed.

Table 2 shows the mass balance of a single wet-air oxidation run using mouse feces. Once again carbon shows a high recovery percentage, the excess carbon probably results from either variations in the % carbon in feces or inaccuracies in the measurement of carbon streams in WAO effluent. The excess hydrogen in the system was assumed to be forming combustion water and thus demonstrates a full recovery. The oxygen recovery is deceiving because of the large excess of oxygen used in the WAO process. Looking at the total oxygen used in the oxidation (1.80 g) and the recovery of only 1.07 g indicates a sink for oxygen which is not yet explained. Once again, nitrogen exhibits a low recovery (86%) which may be due to difficulties in measuring small quantities of nitrogen compounds or variation in the nitrogen content of feces.

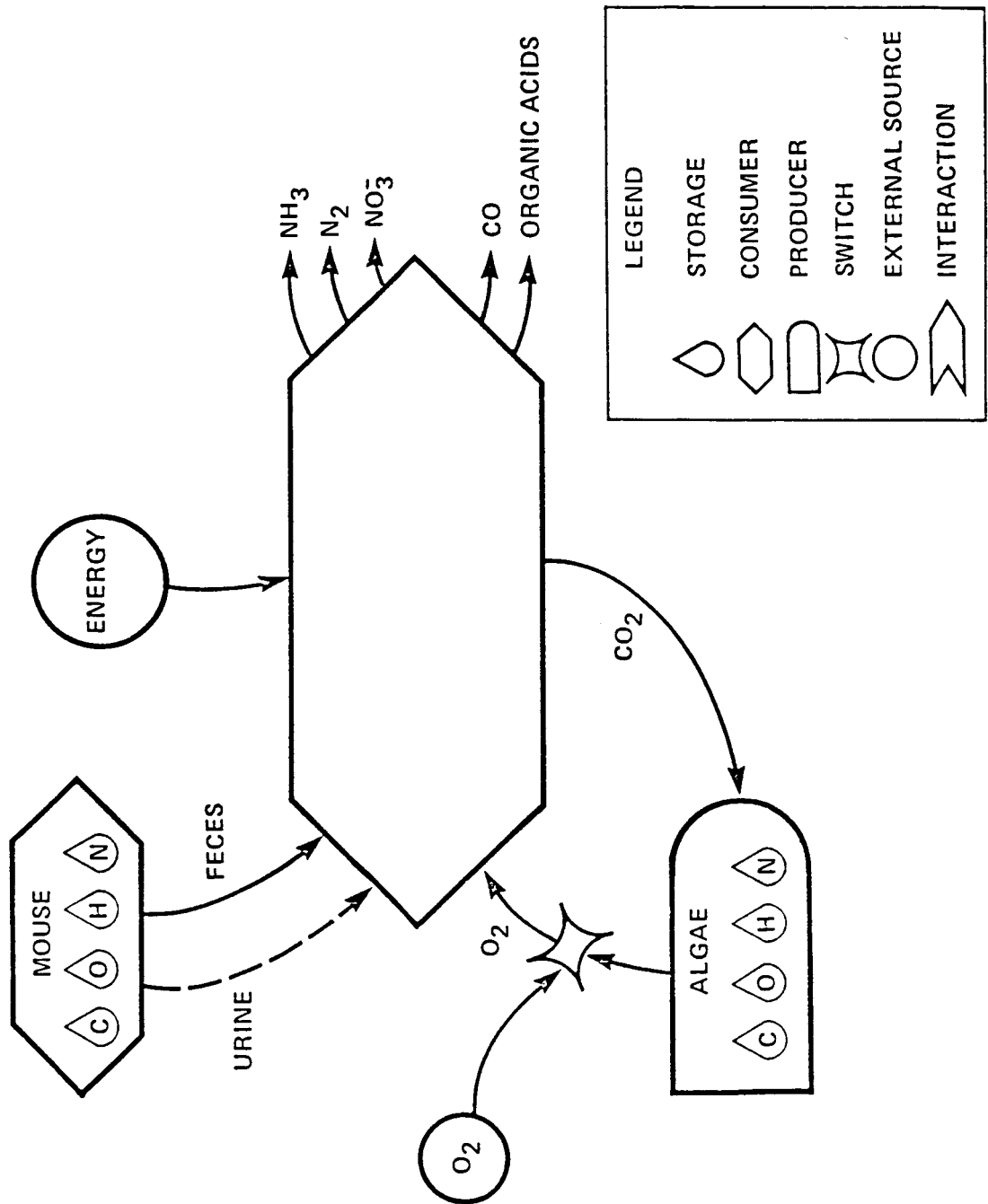


FIGURE 23. SIMULATION MODEL; WET AIR OXIDIZER COMPARTMENT

TABLE 2: WET AIR OXIDATION MASS BALANCE

(All values in grams)

	<u>TOTAL</u>	<u>C</u>	<u>H</u>	<u>O</u>	<u>N</u>
<u>OUT</u>					
Oxygen (g)	22.53	-	-	22.53	-
Carbon Dioxide (g)	0.7806	0.2129	-	0.5677	-
Carbon Dioxide (aq)	0.0118	0.0032	-	0.0086	-
Organic C (aq) (acetic acid)	0.1720	0.0688	0.0115	0.0917	-
Carbon Monoxide	0.2000	0.0857	-	0.1143	-
Nitrogen (g)	0.00444	-	-	-	0.00444
Ammonia (aq)	0.0361	-	0.0064	-	0.02968
Nitrate (aq)	0.00155	-	-	0.0012	0.00035
Organic N(aq)	0.00241	-	?	?	0.00241
Combustion Water	-	-	0.0311	0.2488	-
Precipitate (as Calcium Phosphate)	0.0807	-	-	0.045	-
Total Out	213.74	0.3706	0.049	23.61	0.0369
<u>IN</u>					
Feces	0.934	0.334	0.049	0.289	0.043
O ₂	24.33	-	-	24.33	-
Totals	25.26	0.334	0.049	24.62	0.043
Difference	1.52	-0.0366	0.0	1.057	-0.0061
% Recovery	94.0	110.9	100.0	95.7	85.8

To change the steady-state flow balance of figures 21-23 to a dynamic model, equations must be developed that express the various flows in terms of the state variables, i.e., the various storages of algal and mouse biomass, atmospheric CO₂, O₂, H₂O, light and nutrients. The simulation model being developed allows elemental balances to be calculated from experimental data. When the model is validated it will be useful to observe the changes in mass flow as conditions are varied. Thus, for example, the effects on the system of a doubling of the CO₂ input from the mouse compartment may be done completely by the model without having to actually add a mouse to the experimental system. The storage and flow of elements will be much easier to study with a well validated model than with the experimental system upon which it is based. As the model predicts interesting system behaviors, experiments may be conducted to verify model results and observe actual system behavior. At this time the model is only partially constructed and is not operating on the computer.

Table 3 shows one example of an overall system mass balance used to determine the accumulation and-or depletion of bioelements. The lack of complete experimental data, compounded by the non-integrated nature of the experimental system, makes it difficult to make accurate conclusions about element balance within the system. Data required includes; measurement of water

TABLE 3: MASS BALANCE OF THE MOUSE-ALGAL-WAO SYSTEM

MOUSE (AQ = 0.975)

Start with: 0.5460 g oxygen
which equates to: 2.190 g carbon dioxide produced
1.610 g oxygen consumed

WAO

Requires: 0.602 g oxygen
Yields: 0.4560 g carbon dioxide
0.1838 g carbon monoxide
(+ 0.0668 g oxygen = 0.1838 g carbon dioxide)
0.03633 g nitrogen
(+ 0.1246 g oxygen = 0.1609 g nitrate)

ALGAE

Receives: 2.829 g carbon dioxide
(2.190 + 0.4560 + 0.1838)
Produces: 3.070 g oxygen (AQ = 0.67) experimental
2.110 g oxygen (AQ = 0.975) theoretical

Total Oxygen In: 2.40 g oxygen
(1.610 + 0.0668 + 0.1246 + 0.602)

vapor exhalations, recycling of urine and the measurement of combustion water produced in the WAO.

The data shown in Table 3 indicates that if the AQ is less than the RQ there will be a net increase of O_2 in the system. This agrees with the theoretical calculations shown in Figure 7. If the AQ is equal to the RQ there appears to be a net loss of O_2 although a balance would be expected. The reason for this discrepancy is probably due to the formation of combustion water in the WAO. There is a significant loss of O_2 from the WAO mass balance which, if completely accounted for, would probably balance the O_2 in the AQ equal to RQ scenario of Table 3. When looking at the whole system mass balance, the algal AQ must actually balance with the mouse RQ as well as the WAO RQ (which would equal the ratio of CO_2 produced/ O_2 consumed).

The data in Table 3 exhibits the amount of NO_3^- which would be obtained from the oxidation of the specified amount of feces, assuming the complete reduction of all nitrogen to NO_3^- . This value could then be compared to the amount of NO_3^- required by the algal cultures to support one mouse for one day. Again, data is lacking to make this complete comparison because of the non-integrated nature of the system. However, it is clear that such analyses could be made with a combination of the simulation model and system modifications. Calculation of such balances at this time is not useful because of the

innaccuracies introduced by the nature of the system. More accurate analysis will be possible with the stoichiometric equations contained in the simulation model and data acquired from integrated subsystems.

Growth Experiments

In conjunction with the mass balance experiments a set of experiments concerned with the growth of algae on wet-oxidized mouse feces have been conducted. The purpose of these experiments is to demonstrate that algal growth can be supported by the fecal wet-oxidate. Magnitudes of mass flows must be roughly equivalent so that the supply of feces will provide sufficient nutrients to support the algal growth necessary for O_2 regeneration (and possible food production) sufficient for the mouse supplying the feces. The experimental data are also required to demonstrate the practical application of the wet-oxidation process to the support of algal growth.

Figure 24 shows the resulting growth curves for a control, a 1:1 dilution, a 1:15 dilution and a 1:150 dilution of fecal wet-oxidate. There is inhibition of growth with the 1:1 dilution of fecal oxidate. However, further dilution (1:15 and 1:150) of the oxidate demonstrated that growth can occur at levels equal to the control grown on standard media indicating that at least the minimal nutrient species are present in the oxidate at

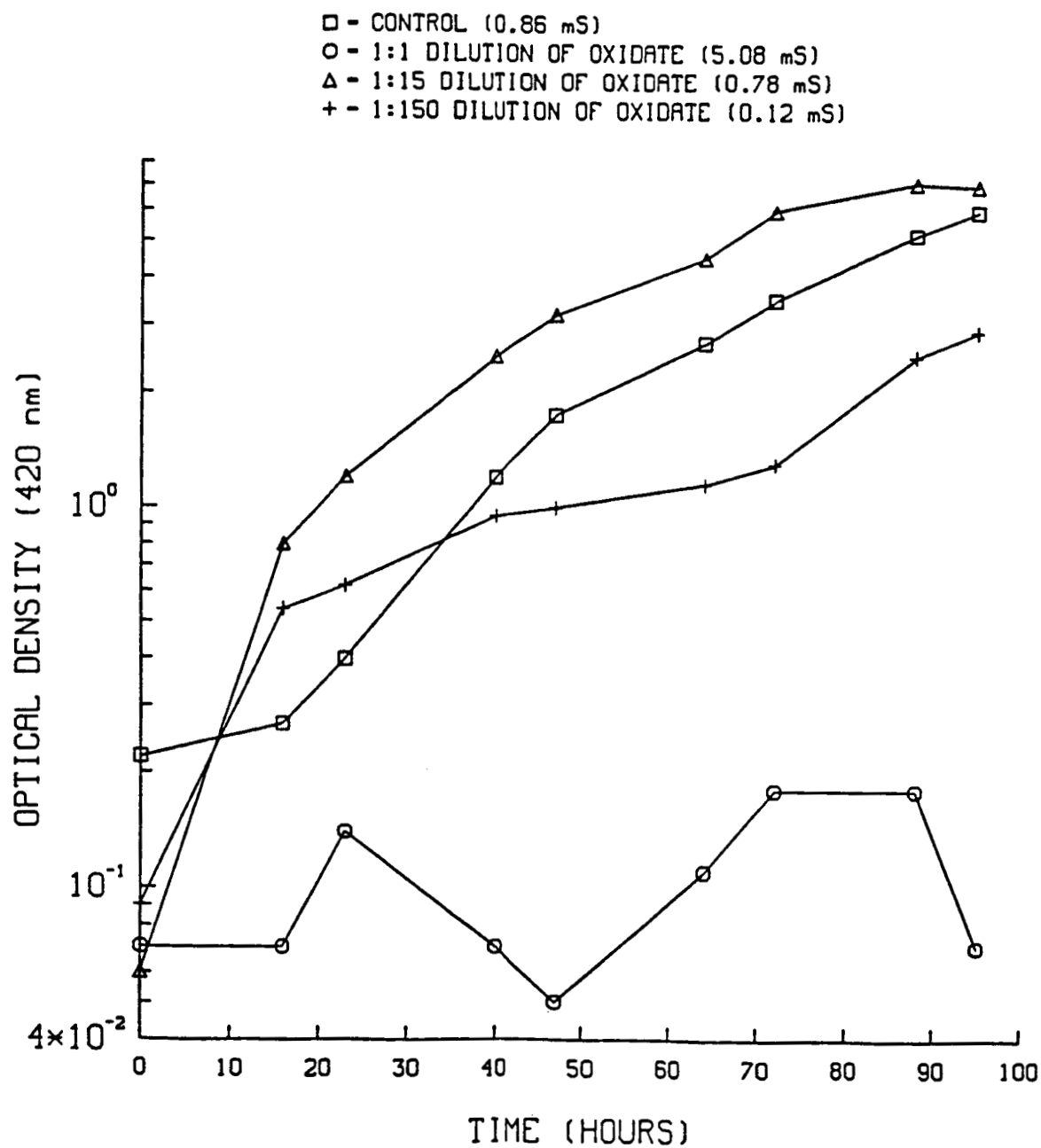


FIGURE 24. GROWTH OF ALGAE ON WET-OXIDIZED MOUSE FECES

sufficient concentrations to support algal growth. CO₂ is supplied to the flasks from tanks but in a physically coupled system would be supplied from the wet-oxidizer or from the mouse.

Table 4 outlines the results of a set of experiments designed to explain the inhibitory nature of the 1:1 dilution of fecal wet-oxidate. The osmolarity of the concentrated oxidate is significantly higher than the standard media. Preparations of media were made with increasing concentrations of nutrient salts. Inhibition of algal growth does not occur with high salt concentration media. It is probable that the inhibitory nature of the oxidate is not due to disruption of algal cell structure or metabolism by high osmolarities.

TABLE 4: EFFECT OF SALT CONCENTRATION ON ALGAL GROWTH

Fecal Wet-Oxidate		Standard Medium	
-----		-----	
MilliSiemens	Growth	MilliSiemens	Growth
-----		-----	
0.86	+	0.86	+
0.76	+	1.06	+
5.08	-	5.00	+

Work is continuing to determine the nature of the inhibitory material(s). Previous related research (Onisko and Wydeven, 1983) indicated the presence of silver and boron in human feces wet-oxidate as a contaminant from reactor conditions. Modifications to the WAO were made and the contamination of reactor products by silver and boron was eliminated. Inhibition in the human feces experiments was determined by toxicity studies on lettuce seedlings. Inhibition studies for the mouse fecal wet-oxidate have not yet looked at trace contaminant effects. The nature of algal growth is different from lettuce seedlings and thus conclusions between the two are difficult to make. The inhibition of algal growth must be further examined before fecal wet-oxidate can be used as a sole source of algal nutrient.

Additional growth experiments have been conducted using lyophilized algal cells and spent nutrient media as inputs to the wet-oxidizer. The results of these experiments indicate that growth at a 1:1 dilution of oxidate is equal to the controls but appears to be slightly inhibited at a 1:10 dilution (Fig. 25). The preliminary conclusions are that at least one essential nutrient is partially limiting at a 1:10 dilution of the algal oxidate. This apparent inhibition by dilution of an essential nutrient also implicates the feces as the source of the fecal oxidate inhibition because it shows a dilution of toxicity and not a dilution of nutrients.

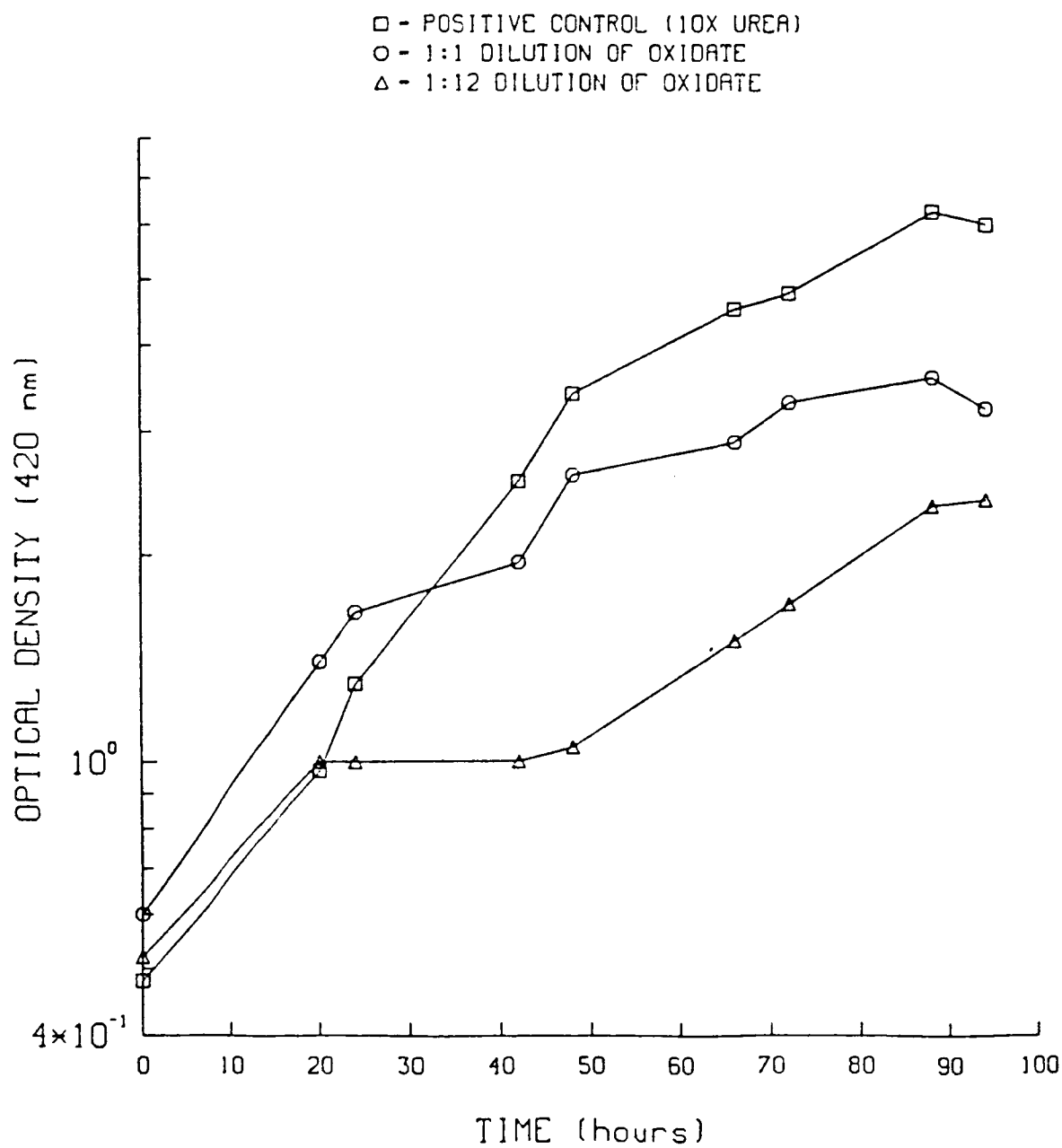


FIGURE 25. GROWTH OF ALGAE ON WET-OXIDIZED CHLORELLA

This idea is supported by the fact that a wet-oxidizer run using only distilled water showed no inhibition of algal growth indicating that the process is not the source of toxicity. Coupled to the fact the algal oxidate does not demonstrate toxicity but rather inhibition by nutrient dilution implicates the feces as a source of the observed inhibition at concentrated levels of oxidate.

Further research is required to definitely identify the inhibitory nature of the fecal oxidate. Although growth can be sustained on dilute fecal oxidate it is not clear what the long term growth characteristics of algae will be on a fecal oxidate diet. Use of oxidate as the sole source of algal nutrients will be conducted in the reactors to determine the growth characteristics in continuous culture. Additionally, the use of algal cells in the WAO will be replaced with the extracted non-edible fraction to better simulate the waste stream anticipated in an operational CELSS.

V. CONCLUSIONS

The need to minimize mass, power and volume requirements of regenerative life support systems has prompted the study of organisms as integral elements of such systems. In order to create a stable spacecraft ecology control over biological systems must be exerted. Approaches to achieve necessary control is a major issue of this research effort. One advantage of using organisms will be the use of their inherent characteristics as control mechanisms in overall system operations. The research described in this thesis has demonstrated that endogenous control strategies based on inherent algal growth characteristics can be successfully applied to the problem of maintaining stable atmospheric concentrations of CO_2 and O_2 within simple closed systems.

There are inherent instabilities in the CO_2 and O_2 concentrations within the gas-closed mouse-algal system. Control of the combined mouse-algal system depends upon the degree of control which can be exerted on the algal component. Algal controls which have been addressed by this research are; variation of light intensity, optical density and nitrogen source. Bacterial populations have also been found to impact algal gas exchange characteristics. Control techniques which

apply but are not covered by this research include the effects of temperature variation, use of multiple autotroph species and variation of nitrogen species concentration.

Variation of light intensity has been shown to alter the algal photosynthetic rate, and may also alter the AQ. Cultures grown on either urea or nitrate exhibit relatively stable concentrations of CO_2 and O_2 if the proper light intensity and optical density are combined. Monitoring of gas concentrations and the adjustment of light levels will have to be done continuously in order to prevent concentrations from varying beyond acceptable limits.

Variation of the optical density of algal cultures has also been shown to effect the gas exchange dynamics of the system. In fact, the relationship between optical density and light intensity may be a key point for controlling respiratory gas concentrations within the system. Further experimental work will make it possible to predict system behavior at specified optical densities and light intensities. The selection of growth conditions which exhibit desired system states will allow control to be exerted with relatively less input of energy and manpower.

Use of different nitrogen sources in algal growth media has demonstrated different AQ's. The manipulation of AQ to more closely match RQ has been shown to reduce atmospheric instabilities within the system. Control of algal AQ has allowed relatively long-term gas-stable operation of the system without the need for other control mechanisms. Still, a combination of AQ variation and optimal light intensity-optical density regimes will result in the most stable system states. Smaller differences in the AQ-RQ mismatch will require relatively less manipulation of the light intensity-optical density regime required to maintain the system within specified $[CO_2]$ and $[O_2]$.

The impact of bacterial populations on system dynamics is believed to vary with relative population sizes. Further work will determine exactly how AQ varies with the magnitude and speciation of bacterial contamination. Reduction of bacterial populations appears to be feasible although complete elimination of bacteria within algal cultures is not feasible for long durations.

There are many algal species and growth conditions which have yet to be fully explored. It will be useful to examine a number of species and conditions in order to design a flexible system able to meet varying atmospheric conditions. As crew oxygen demands vary it will be important for the atmosphere

control system to respond to those changes with a minimal amount of external control. A system which can vary according to the inherent properties of the organisms it contains will require much less control energy than a system which must rely on active control measures.

In fact, the degree of control demonstrated may be greater than is required for atmosphere stabilization. Exogenous controls such as light intensity variation demonstrated the capability to control the atmosphere of the experimental system. Even more crude controls such as venting of excess gases may suffice to maintain appropriate gas concentrations within a space based system. Nevertheless, the reductions in resupply and control energy may improve the feasibility of the overall system. Additionally, the demonstration of endogenous control may find application to other biological systems within a CELSS. Areas less easily controlled by "brute force" methods may benefit from the application of endogenous control strategies.

The maintenance of stable gas concentrations will not suffice for long-term recycling of carbon and oxygen within the experimental system. To achieve this, the food loop of the system must be closed. The preliminary mass balance described herein is limited by the non-integrated portions of the system and the use of external sources of materials.

However, mass balance calculations do suggest that a near complete balance of bioelements may be achieved in a fully integrated system which does not rely upon external supplies.

Calculations of mass flow within the system have shown that the orders of magnitude of flows between system compartments are equivalent. The demonstration of quantitative recycling of bio-elements will require system improvement and expanded data collection. The use of the simulation model will improve the analysis of data and allow more rapid exploration of system dynamics under a variety of operating conditions.

The preliminary mass flow calculations show that the balancing of AQ to RQ is important to atmospheric stability because it reduces control requirements. System mass balance is also apparently improved by close AQ to RQ matching although more data is needed to verify this. The simulation model will permit the analysis of such factors as multiple species and the effects of a variety of operating conditions.

Finally, the development of a non-integrated, partially closed ecosystem has raised a variety of important questions concerning the development of biologically based life support systems for use in space and the application of such information to the management of terrestrial ecosystems. One important question concerns what the effects of closure, per

se, are on the system and its components. This would include the accumulation of biogenic and non-biogenic toxins and the ability of organisms to survive for long periods on completely recycled materials. Another important question is how subsystem control mechanisms can be incorporated into an integrated system. Specifically, how will endogenous control of AQ fit into the waste processing, food production and system control aspects of a CELSS? The answers to such questions must await the development of an integrated set of CELSS subsystems.

The maintenance of terrestrial ecosystems has been left to the natural processes which evolved concurrently with life on the Earth. The impact of our technological society on ecosystems is clearly significant although mechanisms and outcomes of those impacts are not as clear. The study of simple controlled ecosystems will improve our ability to predict and, perhaps, mitigate such impacts. The information contained in this thesis does not significantly enhance the understanding of ecosystem dynamics or the controls which govern them. However, the continued development of controlled ecological systems will improve our understanding of how ecosystems function and how it may be possible to exert control over them or, at least, to improve the strategies by which we can protect them from destruction.

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TABLE 5: MOUSE MASS FLOW DATA

No. Mice	Food g/mouse-day	Feces g/m-d	Water ml/m-d	Urine ml/m-d	Avg. Mouse Weight	Weight Change m-d
1	1.713	0.520	1.795	0.2242	9.542	-0.141
1	1.739	0.4078	2.182	0.6546	9.529	-0.213
1	1.698	0.4791	3.529	0.8229	9.524	-0.291
1	2.165	0.5451	3.364	0.7844	9.768	+0.047
1	2.237	0.6991	3.675	0.9733	9.723	+0.3516
3	1.487	0.4078	2.399	0.8723	10.120	+0.2102
3	1.629	0.4880	2.472	0.7636	10.021	+0.8534
4	1.418	0.4789	2.161	0.5971	9.935	+0.4737
3	1.670	0.5999	2.868	1.320	10.049	+0.3082
3	1.594	0.6101	2.580	1.037	10.519	-0.2278
4	1.296	0.4683	2.105	0.6316	10.270	-0.229
3	1.794	0.6872	3.155	1.690	10.890	+0.0129
4	1.405	0.5058	2.208	0.7641	10.807	-0.0702
3	1.943	0.7275	3.394	1.293	11.007	+0.7764
4	1.465	0.6548	2.315	0.6089	10.758	+0.0282
Mean	1.684	0.5460	2.680	0.8691	10.164	+0.1260
Standard Deviation	0.270	0.1009	0.6014	0.3573	0.520	0.3628

TABLE 6: OD and AQ of NITRATE GROWN CHLORELLA
(350 microeinsteins/m2/s)

CONTAMINATED

RUN	OD	AQ
1	0.32	0.51
2	0.54	0.54
3	0.80	0.70
4	2.0	0.60
5	0.19	0.45
6	0.5	0.48
7	0.80	0.51
8	1.05	0.56
9	0.19	0.50
10	0.24	0.44
11	0.44	0.53
12	1.0	0.43
13	1.2	0.55
14	0.21	0.45
15	0.61	0.45
16	1.2	0.53
17	0.22	0.47
18	0.32	0.37
19	0.41	0.46
20	0.11	0.45
21	0.19	0.57
22	0.24	0.57
23	0.49	0.38
24	1.05	0.40
25	1.30	0.55
MEAN	-	0.50
STANDARD DEVIATION	-	0.07

UNCONTAMINATED

RUN	OD	AQ
1	0.325	0.68
2	1.80	0.69
3	0.56	0.62
4	1.40	0.68
5	1.70	0.66
MEAN	-	0.67
STANDARD DEVIATION	-	0.028

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1. Report No. NASA CR- 177402		2. Government Accession No.		3. Recipient's Catalog No.	
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16. Abstract Life support systems based on bioregeneration rely on the control and manipulation of organisms. Experiments conducted with a gas-closed mouse-algal system designed to investigate principles of photosynthetic gas exchange focus primarily on observing gas exchange phenomena under varying algal environmental conditions and secondarily on studying element cycling through compartments of the experimental system. Inherent instabilities exist between the uptake and release of carbon dioxide (CO ₂) and oxygen (O ₂) by the mouse and algae. Variations in light intensity and cell density alter the photosynthetic rate of the algae and enable maintenance of physiologic concentrations of CO ₂ and O ₂ . Different nitrogen sources (urea and nitrate) result in different algal assimilatory quotients (AQ). Combinations of photosynthetic rate and AQ ratio manipulations have been examined for their potential in stabilizing atmospheric gas concentrations in the gas-closed algal-mouse system. Elemental mass balances through the experimental systems compartments are being studied with the concurrent developoment of a mathematical simulation model. Element cycling experiments include quantification of elemental flows through system compartments and wet oxidation of system waste materials for use as an algal nutrient source. Oxidized waste products demonstrate inhibitory properties although dilution has been shown to allow normal growth. Characterization of the nature of the inhibitory material has begun.					
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